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ABSTRACT BOOK

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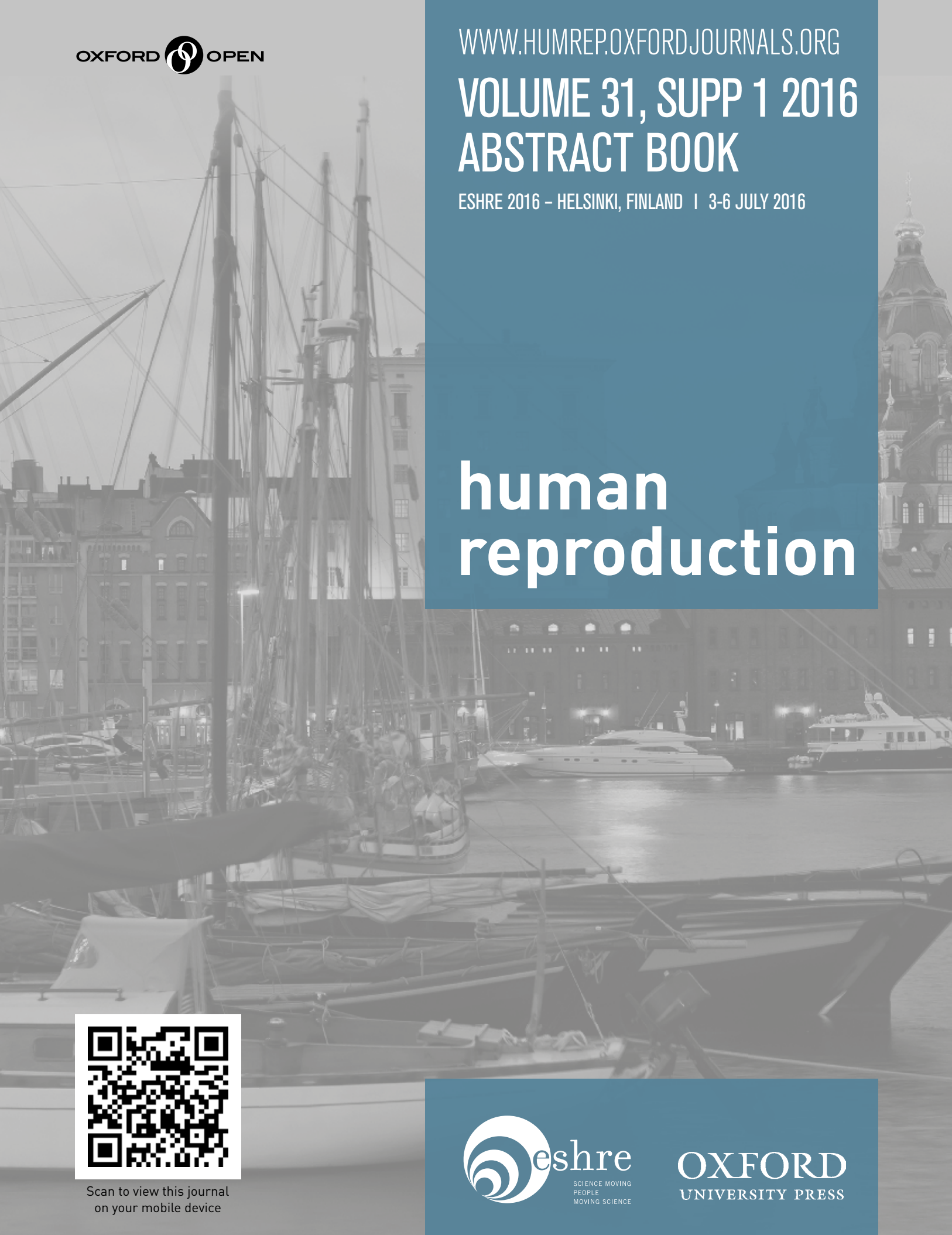

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Abstracts of the 32nd Annual Meeting of the European Society of Human Reproduction and Embryology

Helsinki

Finland

3 to 6 July 2016

Abstracts

32nd Annual Meeting of the
European Society of
Human Reproduction and Embryology
Helsinki, Finland
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The cover of *Human Reproduction* shows histone acetylation in two human germinal vesicle (GV) stage oocytes. The upper panels show an early-stage GV oocyte with a non-surrounding nucleolus stained for (A) chromatin (DAPI; blue) and (B) histone acetylation (anti-H4K12ac; red). Note the regions of intense chromatin staining in some areas, whereas others show no acetylation (C; overlay). The lower panels, of a more developed oocyte with a surrounding nucleus stained for chromatin (D) and histone acetylation (E), show more condensed chromatin than in the early-stage oocyte (above), although the oocyte still has some acetylated chromatin as shown in E and overlay (F). For more details see van den Berg *et al.*, pp. 1181–1190.

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Oral Presentations

INVITED SESSION

SESSION 01: KEYNOTE SESSION

Monday 04 July 2016 **Hall 1** **08:30–09:30**

O-001 Human reproduction keynote lecture – Modifiable and non-modifiable risk factors for poor sperm morphology

A.A. Pacey[1]
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Abstract text
The Chemicals and Pregnancy Study UK (CHAPS-UK) is a comprehensive investigation of >2,200 men attending 12 fertility clinics across the UK for their first semen analysis. In two papers published in Human Reproduction in 2012 and 2015, the data was used to investigate how modifiable and non-modifiable lifestyle factors were related to low motile sperm concentration (<12 million motile sperm per ml) and low sperm morphology (<4% normal forms), respectively. This is important because whilst many studies have claimed that men's lifestyle can affect semen quality, the evidence is weak with studies often underpowered and poorly controlled. The design of CHAPS-UK addresses many of these criticisms recruited eligible men, aged 18 years or above, were part of a couple who had been attempting conception without success following at least 12 months of unprotected intercourse and also had no knowledge of any semen analysis before being enrolled.

In our 2012 paper we found that risk factors for low motile sperm concentration, after adjustment for recruitment centre and confounding factors, included a history of testicular surgery [odds ratio = 2.39, 95% confidence interval (CI): 1.75, 3.28], being in manual work [odds ratio (OR) = 1.28, 95% CI: 1.07, 1.53] or not working (OR = 1.78, 95% CI: 1.22, 2.59) and having black ethnicity (OR = 1.99, 95% CI: 1.10, 3.63). Conversely, men who wore boxer shorts (OR = 0.76, 95% CI: 0.64, 0.92) or who had a previous conception (OR = 0.71, 95% CI: 0.60, 0.85) were less likely to be a case. No significant association was found with smoking and alcohol consumption, the use of recreational drugs, a high BMI or having a history of mumps or fever. By contrast, in our 2015 paper we found that risk factors for poor sperm morphology, after adjustment for centre and other risk factors, included: (i) sample production in summer (odds ratio (OR) = 1.99, 95% confidence interval (CI) 1.43–2.72); and (ii) use of cannabis in the 3 months prior to sample collection in men aged ≤30 years (OR = 1.94, 95% CI 1.05 – 3.60). Men who produced a sample after 6 days abstinence were less likely to be a case (OR = 0.64, 95% CI 0.43–0.95). No significant association was found with BMI, type of underwear, smoking or alcohol consumption, or having a history of mumps.

For both analyses, the data had been collected blind to outcome and so exposure information should not have been subject to reporting bias. This is a major strength of the study. In conclusion, both of our analyses of the CHAPS-UK data suggest that common lifestyle choices generally make little contribution to either low motile sperm concentration or low sperm morphology. Therefore, any delay to assisted conception in order to make poorly evidenced changes to lifestyle to improve semen quality is not recommended.

O-002 Long-term consequences of maternal obesity on the health of offspring

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Abstract text
The Developmental Origins of Health and Disease (DOHaD) hypothesis proposes that several non-communicable diseases – including coronary heart disease and type 2 diabetes – have their origins in prenatal life and in early childhood. The intrauterine *milieu* which is influenced by a large number of factors – including maternal characteristics – affects the developing fetus *via* a number of pathways resulting in the programming of future health outcomes.

Early life programming has mostly been studied in relation to long-term health outcomes in relation to being born with a small body size. However, several more recent studies have been reporting associations between maternal obesity and later health outcomes in the offspring. The prevalence of overweight and obesity are increasing worldwide and within the European Union about one-third of women of reproductive age are overweight, and every fifth is obese. Maternal obesity is associated with immediate adverse maternal and neonatal outcomes including an increased risk of congenital defects and miscarriage. Further there is increasing evidence suggesting that maternal obesity also has long-term consequences for the offspring's later health and wellbeing.

Maternal obesity in pregnancy has been associated with an increased risk of premature death in adult offspring. Further based upon findings from the Helsinki Birth Cohort Study it has been shown that higher maternal pregnancy BMI was associated with an increased risk of cancer, cardiovascular disease, and type 2 diabetes, among the offspring. The association with type 2 diabetes was stronger in women, consistent with the transmission of type 2 diabetes from the mother to her daughters being stronger than transmission to her sons.

Maternal BMI was positively associated with BMI in the offspring as expected. Higher maternal BMI was associated with less favorable body composition in the offspring. There was a significant interaction between birth weight and maternal BMI on offspring body fat percentage in adult life. In mothers with low BMI, a higher offspring birth weight was associated with lower fat percentage, while among those with maternal BMI in the highest fourth, higher offspring birth weight predicted higher body fat percentage in adult life. Our findings suggest that a disadvantageous body composition is programmed in early life, as a consequence of prenatal growth and maternal adiposity. This may in part underlie the association between maternal obesity and later cardiometabolic health in the offspring. These findings support the importance of prevention of overweight in women of childbearing age.

One plausible explanation for an association between maternal obesity, which provides adverse intrauterine experiences, and later health is in utero programming, which may work through environmental, genetic, and epigenetic mechanisms.

SELECTED ORAL COMMUNICATIONS

SESSION 02: OOCYTE HANDLING/ACTIVATION AND EMBRYO CULTURE

Monday 04 July 2016 **Hall 1** **10:00–11:30**

O-003 Influence of the duration between removal of cumulus cells and oocyte retrieval on fertilization and embryonic development

Y. Ishikawa[1], M. Inaba[1], H. Matsumoto[1], S. Mizuno[1], R. Mori[1], M. Ida[1], A. Fukuda[1], Y. Morimoto[2]
[1]*IVF Osaka Clinic, Department of Ob/Gyn, Osaka, Japan*
[2]*HORAC Grand Front Osaka Clinic, Osaka, Japan*

Study question: The aim of present study was to investigate if the timing of cumulus removal post retrieval would be a critical on fertilization and embryonic development.
Summary answer: The present study suggested sufficient time to culture oocytes with intact cumulus cells post-OPU have favorable influence on the embryonic development compared to immediate denudation.
What is known already: The role of cumulus cells surrounding oocytes in the process of maturation, ovulation, and fertilization in mouse has been extensively studied. Prolonged culture of oocyte with intact cumulus cells has been reported to induce apoptotic changes in oocytes. However, the influence of culture duration of oocyte with cumulus cells in human has not been investigated not only on competence of oocyte, but also subsequent embryonic development.
Study design, size, duration: Prospectively randomized study was performed on 667 oocytes retrieved from 54 patients (54 cycles) between October 2013 and May 2015.

Participants/materials, setting, methods: Patients under 39 year old treated by ICSI with controlled ovarian stimulation were randomly divided into 2 groups. Cumulus cells were removed immediately after oocyte retrieval in group A and were removed 120 min after retrieval in group B. ICSI was performed on all matured oocytes and they were cultured to blastocyst stage. The rates of maturation, fertilization, abnormal fertilization, blastocyst and good quality blastocyst were compared between the two groups.
Main results and the role of chance: The rates of maturation, fertilization, abnormal fertilization, and blastocyst between group A and B were 84.2 ± 13.0% vs. 88.1 ± 11.3%, 88.4 ± 10.2% vs. 88.3 ± 11.4%, 5.6 ± 7.3% vs. 4.9 ± 8.4%, 62.8 ± 30.1% vs. 73.8 ± 23.3%, respectively and no significant differences in any category. On the other hand, there was significant difference ($p < 0.05$) in good quality blastocyst rates (33.1 ± 28.4% vs. 52.0 ± 30.9%) between group A and B.
Limitations, reasons for caution: None.
Wider implications of the findings: The present study revealed that culture of oocyte with cumulus cells 2 h or longer improved the quality of resulting blastocyst. Time lag between ICSI and oocyte retrieval might be required to acquire sufficient maturation on cytoplasm of oocyte and beneficial to achieve pregnancy in IVF practice.
Trial registration number: None.

O-004 Late activation with calcium ionophore is associated with chromosome retention by the oocyte following completion of meiosis II and extrusion of the second polar body

R. Blanshard[1], C. Ottolini[2], A. Capalbo[3], S. Natesan[4], A. Handyside[4], E. Hoffmann[1]
[1]*MRC Genome Damage and Stability Centre, Life Sciences, Brighton, UK*
[2]*The Bridge Centre, London, UK*
[3]*GENERA Gynecology-Endocrinology and Assisted Reproduction, Centers for Reproductive Medicine, Rome, Italy*
[4]*Illumina, Reproductive and Genetic Health, Cambridge, UK*

Study question: Does artificial oocyte activation (AOA) with calcium ionophore affect the segregation of chromosomes in the second meiotic division (MII)?
Summary answer: Oocytes activated following a second ionophore exposure, 24 h after initial treatment, were associated with retention of both copies of one or multiple chromosomes at MII.
What is known already: Artificial oocyte activation (AOA) by exposure to calcium ionophore is being increasingly used to overcome partial or complete fertilisation failure after intracytoplasmic sperm injection (ICSI). SNP genotyping and MeioMap analysis of all three products of female meiosis, the first and second polar bodies (PB1 and PB2) and corresponding activated oocytes, has identified six different patterns of chromosome segregation errors. Recently, we used MeioMap analysis in a pilot study of activated oocytes to assess the effects of exposure to calcium ionophore A23187, and found no widespread increase in segregation errors in meiosis II.
Study design, size, duration: Maternal genomic DNA and whole genome amplified products from artificially activated oocytes together with both matching polar bodies, were genotyped for ~300,000 SNPs genome-wide. Informative maternal heterozygous SNPs were phased using a PB2 or oocyte as a reference and the two maternal haplotypes mapped (MeioMapped) in each of the samples, allowing identification of chromosome segregation errors in the first and second meiotic divisions (meiosis I and II).
Participants/materials, setting, methods: With the patients' informed consent, surplus vitrified oocytes arrested at metaphase of meiosis II were thawed and the PB1 was biopsied. The oocytes were then exposed to calcium ionophore for AOA. After resumption of meiosis and extrusion of the PB2, the PB2 was biopsied and the activated oocytes were isolated. Both polar bodies and the activated oocytes were subjected to whole-genome multiple displacement amplification and SNP genotyping by microarray.
Main results and the role of chance: Ten vitrified and thawed MII arrested oocytes from two patients were exposed to calcium ionophore A23187 for AOA. All four oocytes from one patient (maternal age 31 years) but only one out of six oocytes from the second patient (maternal age 29 years) activated and extruded the PB2. The non-activated oocytes were therefore re-exposed to the ionophore 24 h later, of which four then activated.

In the five oocytes which activated after the initial exposure to ionophore, only one chromosome segregation error was identified by MeioMapping resulting in monosomy 17 in the activated oocyte due to non-disjunction at meiosis II. An example of reverse segregation, in which both homologues segregate sister chromatids to both PB1 and oocyte, was also identified for chromosome 15 in a second oocyte; however, the two chromatids segregated normally in meiosis II.

In contrast, one of the oocytes which only activated after a second treatment with ionophore, retained both chromatids in the oocyte for 14 out of 23 pairs. Furthermore, there were five other meiosis II errors totalling 19 out of 115 pairs, all resulting in chromatid retention in the oocyte, which would not be expected by random non-disjunction events ($p = 0.0002$)
Limitations, reasons for caution: The current work is a pilot study of a small number of activated oocytes. Further work is needed to ascertain if retention of chromosomes at meiosis II is caused by repeated exposure to calcium ionophore, or whether the timing of exposure is critical.
Wider implications of the findings: MeioMapping is a powerful tool for the preclinical assessment of novel treatments which may affect human oocytes, and can accurately identify and distinguish abnormalities arising in both meiotic divisions. This is important since, in ART settings, meiosis I is normally completed *in vivo* whereas meiosis II occurs *in vitro*.
Trial registration number: RB, SN and AH (part time) are employed by Illumina (Cambridge, UK), which provided support and a studentship (RB) for the research.

O-005 A prospective randomized controlled trial investigating embryonic development and clinical outcome after using $Ca2^+$ ionophore in cases with previous fertilization arrest

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[1]*Cairo, Egypt*
[2]*TopLab Co. for ART Laboratories' Consultation and Training, Research and Development, Cairo, Egypt*

Study question: Can A23187 $Ca2^+$ ionophore improve embryonic development in patients with a history of complete fertilization arrest and inability to transit to cleavage stage after ICSI?
Summary answer: In patients with previous history of complete fertilization arrest, using $Ca2^+$ ionophore activation may improve embryonic development which, in turn, may improve clinical outcome measures.
What is known already: Various studies have shown that $Ca2^+$ ionophore activation improved ICSI outcomes in cases with total fertilization failure after ICSI. However, the effect of $Ca2^+$ ionophore activation in selected cases with cleavage failure after successful fertilization was only discussed in a preliminary study that reported a beneficial effect of $Ca2^+$ ionophore activation in reaching more progressed embryonic developmental stages in four women with a history of complete fertilization arrest and inability to transit to cleavage stage during previous ICSI attempts. This study also showed one successful clinical pregnancy ends with delivery of two healthy livebirths with no malformations.
Study design, size, duration: This randomized controlled trial was carried out at a private fertility center between May 2012 and December 2015. This study included 50 ICSI cycles of patients with a history of complete fertilization arrest after conventional ICSI which were randomized into equal two groups. Patients underwent either another conventional ICSI or ICSI combined with $Ca2^+$ ionophore oocyte activation.
Participants/materials, setting, methods: All patients underwent controlled ovarian stimulation with the GnRH agonist and recombinant FSH in a long protocol. In With group, injected oocytes were cultured in 200 µL of incubated $Ca2^+$ ionophore for 15 min after ICSI. All embryo transfers were performed on Day 5. The primary outcomes are the percentage of cleaved embryos on day 3 and blastocysts on day 5. Secondary outcomes were utilization rate, pregnancy and implantation rates.
Main results and the role of chance: There were no statistical differences between Wout group and With group regarding age (30.42 ± 3.89 and 31.92 ± 3.57 years, respectively), female BMI (25.65 ± 5.05 and 25.85 ± 4.8 Kg/m, respectively), duration of infertility (7.52 ± 2.63 and 8.02 ± 2.51 years, respectively), endometrial thickness (10.2 2.09 and 9.7 ± 2.31 mm, respectively), days of stimulation (9.5 ± 0.73 and 9.7 ± 0.65 days, respectively), peak E2 (2611 ± 1279.4 and 2395 ± 1100 pg/ml, respectively), and peak P4 (0.89 ± 0.52 and 0.92 ± 0.48 ng/ml, respectively). The two groups also were similar in the number of oocyte retrieved, and the number of injected MII oocytes. Fertilization 53.23% vs. 72.56%, cleavage 31.7% vs. 82.9%, and blastocyst formation rates 22.3%

vs. 38.7% were statistically different in Wout and With groups, respectively ($p < 0.001$). ICSI cancellation rate was higher in Wout group (28%) as compared to With group (4%), ($p < 0.001$). Due to the difference in blastocyst formation rate between both groups, the mean number of transferred embryos was lower in Wout group as compared to With group. In the With group, a 41.67% clinical pregnancy rate was observed while in the Wout group only 11.1% ($p < 0.001$). Significant differences were observed between the Wout group and With group regarding Implantation rate (13.04% vs. 39.13%).

Limitations, reasons for caution: Small sample size of the study participants is due to the rarity of such cases. Larger prospective trials with an increased number of patients are needed to confirm our findings.

Wider implications of the findings: According to our results we could suggest that $Ca2^{+}$ ionophore activation may provide a useful tool to improve ICSI outcomes in a selected category of patients suffering from previous fertilization arrest after conventional ICSI. Our interesting finding offers a new area for further research.

Trial registration number: NCT02683031

O-006 Shift in pH during transition to the embryonic genome impacts embryo development

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[1]*Frisco Institute for Reproductive Medicine, Frisco IVF, Frisco, TX, USA*

Study question: Will embryos exposed to a pHe shift during the transition to the embryonic genome develop differently?

Summary answer: The pH shift impacts embryo development. With alkaline–acid shift, the embryos develop slower than controls. With acid–alkaline shift, development is faster.

What is known already: Internal pH (pHi) of embryos is ~ 7.15. When external pH (pHe) changes, pHi of the embryo initially follows. Efforts to keep pHi stable utilizes resources. Even if embryos can survive outside the optimum pHe, quality may be impacted. Some *in vivo* measurements indicate an alkaline–acid gradient for the embryo within the female reproductive system. However, coculture experiments suggest that ciliated oviductal cells generate a relative acidic environment compared to the uterus. Therefore, this study was designed to test a pHe shift in both directions during the transition from maternal to embryonic genome, compared with a control with no shift.

Study design, size, duration: The study is performed using sibling oocytes from patients undergoing IVF/ICSI treatment. The study is ongoing. In this primary analysis a total of 150 oocytes (9 patients, age 30–35) are included. All embryos were cultured at +37°C/5% O2 in the same model incubator equipped with PrimoVision. The only variable was the CO2 concentration. The pH in experimental groups deviated ~ 0.1 from the control group (pH 7.3)

Participants/materials, setting, methods: Patient zygotes were evenly divided into 3 treatment groups: 1) Control in 6% CO_2 throughout, 2) alkaline–acid shift on day 3–5.5% to 6.5% CO_2 and 3) acid–alkaline shift on day 3–6.5% to 5.5% CO_2. Predetermined time-lapse parameters for each zygote were expressed as hours post insemination. Groups were compared using students *t*-test. Primary endpoints were the time-lapse measurements and secondary endpoint was utilization rate.

Main results and the role of chance: The analyses of the first 150 oocytes show that a pHe shift on day 3 does impact embryo development. Initially, the first cell cleavages (t1–t8) were identical between the three groups. For all parameters past the morula stage (tSB, tB, tEB) zygotes exposed to an acid–alkaline pHe shift developed at a faster rate compared with zygotes exposed to an alkaline–acid pHe shift (e.g., tEB; 110 ± 9 h vs. 120 ± 11 h; $p < 0.05$). Furthermore, after the pHe shift, the alkaline–acid group tended to develop slower ($p = 0.07$) and the acid–alkaline group appear to develop faster (not significant) when compared to the control (115 ± 11 h). Due to the progressive decrease in embryo numbers towards the end of the observation period and the initial lower number of patients in this study, *p*-values did not yet reach full statistical significance when comparing with the control. The utilization rate (number of high-quality blastocysts available for cryopreservation and/or transfer) were identical between the groups. However, the acid–alkaline shifting tended to result in proportionally more blastocysts on day 5 compared to the alkaline–acid group (77% vs. 53% blastocyst formation rate, $p = 0.09$) at this early stage of this study.

Limitations, reasons for caution: Changing the CO_2 of the incubator affects not only pHe but also the concentration of H^{+} molecules available for the molecular reactions or gene expression during maternal-zygote genome transition. The differences observed may therefore not solely be attributed to the pH *per se* but also due to the shift.

Wider implications of the findings: Despite embryos ability to adapt and maintain a stable pHi, it is likely that adaptation have consequences on quality. In this study, pH shifting during the maternal-embryo genomic transition impacts embryo development. These minor deviations, possibly mediated through pH dependent gene expression, may have long-term effects.

Trial registration number: Not applicable.

O-007 Prospective randomized study comparing human embryo development in a microwell group culture dish (Primo Vision dish) or in a standard dish with individual droplets

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Study question: Does embryo culture in the microwell group culture dish improve embryo development and IVF outcome compared to individual microdrop culture in conventional Petridish.

Summary answer: Culture in microwell group culture dish (PrimoVision, Vitrolife) resulted in better fertilization rate, faster embryo development, higher clinical pregnancy rate and better embryo utilization rate.

What is known already: Embryos in human IVF treatment are frequently cultured individually in microdrops allowing individual assessment and follow-up of embryo quality. Culturing more than one embryo in the same microdrop may result in accumulation of autocrine and paracrine factors in culture media having a beneficial effect on embryo development. The microwell group culture dish (Primo Vision dish) contains 9 microwells with a well-to-well distance facilitating paracrine effects and specific well morphology facilitating autocrine effects. The design ensures identification of individual embryos cultured together in the same microdrop.

Study design, size, duration: Five hundred thirty two IVF-ET cycles were enrolled in this prospective randomized study between September 2012 and April 2015. Cycles were randomized using a computer generated table. Oocytes were fertilized by conventional IVF or ICSI. Embryos from 264 cycles were cultured in microwell group culture dish (Study Group) and 268 cycles were allocated into individual cultured (Control) group. Viable embryos were transferred or cryopreserved at cleavage stage.

Participants/materials, setting, methods: IVF cycles were randomized into Study and Control Groups. In the Study Group up to 9 embryos were cultured together in 25 µl culture media. In Control Group embryos were placed into a 25 µl droplet individually. Time-lapse morphology evaluation system was not used in this study, thus embryo development and morphology was assessed by conventional methods. Fertilization rate, embryo morphology, pregnancy rate and embryo utilization rate were compared.

Main results and the role of chance: Cycle characteristics (female age, length of stimulation, gonadotrophin dose, number of oocytes and number of transferred embryos) were similar in the two groups.

Fertilization rate in ICSI cycles – where oocytes were placed into culture dish immediately after sperm injection – was significantly higher in the Study Group compared to the Control Group (70.6% vs. 64.9%, $P = 0.001$). Fertilization rate in conventional IVF cycles was similar in the two groups. Number of blastomeres on Day 3 was also significantly higher in the Study Group compared to Control (7.0 ± 2.2 vs. 6.7 ± 2.3; $P = 0.013$). Clinical pregnancy rate was 50.8% in the Study Group and 40.6% in individual culture ($P = 0.022$). Multiple pregnancy rate was significantly higher in Study Group than in Control Group (28.5% vs. 40.5%; $P = 0.044$). Implantation rate was similar in the two groups (30.1% vs. 27.0%; $P = 0.265$).

A higher proportion of embryos was available for cryopreservation on day 3 in the Study Group (39.7% vs. 32.1%; $P = 0.024$), thus, embryo utilization rate was higher in microwell group culture compared to individual culture (81.3% vs. 74.7%; $P < 0.001$).

Morphology evaluation needed less time in the microwell group culture dish compared to conventional Petridish (144 ± 70 vs. 175 ± 73 s/cycle; $P < 0.001$) which resulted ~ 30 s shorter time outside the incubator.

Limitations, reasons for caution: The higher multiple pregnancy rate in control group reduced the difference in the implantation rate between groups. Further studies are needed to find explanation of the higher multiple pregnancy rate in the control group.
Wider implications of the findings: Using microwell group culture dish has a beneficial effect on IVF outcome and it also allows for individual assessment and following up individual embryo quality. Faster morphology evaluation means shorter time outside the incubator which decreases fluctuation of temperature and pH and also reduces the workload of the embryology laboratories.
Trial registration number: ClinicalTrials.gov: NCT01774006.

O-008 Do not disturb the embryos until day 5: preliminary results of a double blind prospective randomized controlled trial

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Study question: To test the hypothesis that avoiding embryo observation until day 5 may produce an improvement in embryo quality and therefore, implantation and ongoing gestation rates.
Summary answer: Maintaining the embryos to the blastocyst stage without assessment or observation on day 2 and 3 does not affect clinical outcomes.
What is known already: In a conventional *in vitro* fertilization (IVF) cycle, daily microscopic observation of embryos outside the incubator is performed to assess their morphology and establish a selection process. However, these observations can produce deleterious effects on embryo development due to environment changes: temperature, pH and osmolarity of the culture media, as well as a negative effect of direct light microscope for observation. Serial shooting by time-lapse systems could also have a negative effect.
Study design, size, duration: This is a double blind (patients and clinicians), randomized controlled trial approved by an Ethics Committee. The objective is to detect a 10% increase in pregnancy rate in the study group (non-embryonic observation, 586 cycles) compared to the control group (conventional observation on day 2 and 3, 305 cycles) in a 3-year period. We present preliminary results of 130 IVF treatments (study group, 67 and control group, 63).
Participants/materials, setting, methods: Couples enrolled in a first IVF treatment using anonymous oocyte donation. After meeting the inclusion (normal uterine cavity) and exclusion (recurrent miscarriage and repeated implantation failure) criteria, couples signed an informed consent and they were randomized in a control or study group. After checking the fertilization, zygotes were cultured in Global Total medium (LifeGlobal, Canada) in a benchtop incubator (Planer, Origio) until day 5. Blastocyst formation rate and clinical outcomes were evaluated.
Main results and the role of chance: We analysed our preliminary data. The number of donated eggs was similar in both groups (11.3 ± 2.1 in the control group and 11.1 ± 2.0 in the study group). There were no significant differences in the fertilization rate in the control group (78.4%) compared to the study group (75.0%), $p = 0.2$. In terms of the number of embryos that reach blastocyst stage on day 5, the results were also similar between the two groups (60.6% vs. 62.1% in the control and study groups, respectively, $p = 0.7$). The number of embryos transferred was 1.5 ± 0.5 in the control group and 1.4 ± 0.5 in the study group, with no significant difference ($p = 0.1$). Regarding implantation rates, embryos that were evaluated on day 2, 3 and 5, had similar implantation potential than those that were cultured until blastocyst and evaluated at this stage of development (47.4% vs. 44.0%, respectively, $p = 0.7$). Finally, the ongoing pregnancy rate (OPR) until the 28th week of gestation was similar in the two groups, with no statistical significance ($p = 0.7$). In the group of no observation, the OPR was 43.1%, and in the conventional control group, 47.5%.
Limitations, reasons for caution: The results obtained at this moment are preliminary, so we need to continue with the trial to confirm our hypothesis.

It seems that there is no negative effect on clinical outcomes when we ignore the information provided by embryos on day 2 and 3 of development.
Wider implications of the findings: One assessment of embryos on day 5 prior to transfer or freezing, gives at this time of the trial, the same clinical results as the conventional workout. This reduces the cost of the treatments and calls into question the predicting value of morphological and kinetic parameters in early embryo development.
Trial registration number: Clinicaltrials.gov: NCT02372279.

SELECTED ORAL COMMUNICATIONS

SESSION 03: ART IN THE DIFFICULT PATIENT

Monday 04 July 2016 **Hall 5 CB** **10:00–11:30**

O-009 Optimization of the ovarian reserve in Poor Responder patients by ovarian autologous transplantation of mobilized-bone marrow derived stem cells.

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Study question: To evaluate if Bone marrow derived stem cell transplant (BMT) optimizes follicular recruitment in Poor Responder (PR) women and to elucidate the underlying mechanisms.
Summary answer: Forty percent of PR patients increased Antral Follicular Count (AFC) and Anti-Müllerian Hormone (AMH) being this fact associated with soluble factors already present in BMT.
What is known already: Advanced maternal age is a main cause of infertility, in fact both oocyte quantity and quality are seriously impaired in aged patients. These women are known as PR and oocyte donation is their only practical option. Previous studies suggest regenerative effects of BMT in ovarian niche of damaged ovaries and raise the possibility that dormant follicles or somatic cells may benefit from the influence of BM derived cells or soluble factors. This concept is supported by the recovery of fertility in women after BMT. Within BMT, the $CD133^+$ are the most undifferentiated population previously identified at ovaries.
Study design, size, duration: A prospective pilot study with 10 PR women was developed at University and Polytechnic Hospital La Fe between September 2014 and September 2015. Patients were considered as their own control as cells were injected in just one ovary. The contralateral ovary was considered as the control one.
Participants/materials, setting, methods: BM derived stem cells were mobilized to peripheral blood with G-CSF and isolated by apheresis. A volume of aphaeresis containing 5×10^7 $CD133^+$ cells was delivered into one ovarian artery by cathetherism. Serum AMH and AFC were monitored up to 5 months and compared to basal levels.

Soluble factors involved in growth, stem cell signalling and oocyte activation and development as well as the distribution of the stem cell populations contained in BMT were quantified.
Main results and the role of chance: An enhancement of AFC (≥3) in the treated ovary was considered as BMT primary success criteria while two consecutive increases in AMH was the secondary outcome.

BMT improved ovarian reserve function in 40% of the PR patients. Both criteria were accomplished by 30% of the recruited patients while 10% solely reached the AFC increase and 10% the AMH improvement.

Then, the association between AFC and AMH increase and the presence of soluble factors released by BM stem cells was established. Fibroblast Growth Factor-2 (FGF-2) presence in plasma was associated with the improvement in both parameters, being statistically significant for AMH ($r = 0.91$). Thrombospondin (THSP-1) concentration was positively correlated with the enhancement in AFC ($r = 0.98$) while Insulin-like Growth Factor (IGF-1) and Stem Cell Factor (SCF) secretion manifested a negative correlation with this parameter ($r = -0.883$; $r = -0.99$, respectively). Furthermore, SCF level was also associated with AMH ($r = 0.82$).

On the one hand, the $CD133^+CD34^+$ cell population has been proposed as essential to allow BM cell engraftment while the $CD133^+CD34^-$ displayed the most stemness features. Our preliminary data showed a positive influence of higher concentrations of the $CD133^+CD34^+$ cell population on BMT when assessed as AMH improvement, although being not statistically significant ($p = 0.05$).
Limitations, reasons for caution: Pilot study. Confirmation of our preliminary data on a large population should be required.

Wider implications of the findings: We found that autologous BMT using a semi-invasive and easily reproducible technique enhanced AFC and/or AMH levels in PR patients, improving their reproductive potential. This is relevant considering that nowadays oocyte donation is the only practical option for these patients that represent 9–24% of patients attending an ART clinic.
Trial registration number: [NCT02240342]

O-010 Freeze-all in older women: benefit or loss?

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Study question: Does a freeze-all strategy have an impact on reproductive outcomes in women 39 years and older?
Summary answer: A freeze-all strategy yields better clinical pregnancy rates, especially in women of 39 years and older, compared to women receiving a fresh transfer.
What is known already: Currently a freeze-all strategy, i.e., the elective freezing of all embryos from an IVF cycle and cryotransfers at a later time, is mainly employed to prevent ovarian hyperstimulation syndrome (OHSS), or in presence of an unfavorable endometrium. Older IVF patients commonly present a diminished ovarian reserve and sub-optimal oocyte quality. These women usually have their best embryos transferred during a fresh cycle and are therefore exposed to embryo-endometrium asynchrony. Since sub-optimal embryo quality cannot be amended, efforts should be directed towards providing the best endometrial environment for embryos to be transferred to, which might be achieved by a freeze-all strategy.
Study design, size, duration: Retrospective cohort study of 1,697 first embryo transfer cycles, corresponding to 1,469 women, performed between January 2013 and December 2014. Sixty-nine percent ($n = 1,180$) corresponded to fresh embryo transfers and 30.5% were frozen embryo transfer (FET) after a freeze-all strategy. Controlled ovarian hyperstimulation was performed by either a GnRH-antagonist or a long GnRH-agonist protocol. Ovulation was triggered using either a GnRH-agonist ($n = 292$) or hCG ($n = 1,405$).
Participants/materials, setting, methods: Clinical pregnancy rate and miscarriage rates were compared among first embryo transfer, either fresh (FRESH) or frozen (FROZEN). In order to study the effect of freeze-all on older women, data were stratified according to a woman age <39 ($n = 1,056$) or ≥39 years ($n = 641$). Student's *t*-test for independent samples and Chi-square analysis were used. Moreover, logistic regression analyses were performed adjusting for maternal age and number of transferred embryos. A $p < 0.05$ was considered significant.
Main results and the role of chance: Clinical pregnancy rates (CPR) were significantly higher for FROZEN in both age groups. In the <39 years group, CPR for FROZEN vs. FRESH were 44.5% and 38.2%, respectively ($p = 0.044$) and in the ≥39 years group they were 34.9% and 22.7%, respectively ($p = 0.005$). There was no difference in miscarriage rate between groups: 6.4% for FROZEN vs. 7.5% for FRESH in the younger group ($p = 0.49$) and 8.7% vs. 7.6% ($p = 0.66$), respectively, for the older group. The multivariate analysis found a significantly positive effect of performing FROZEN on CPR in both age groups, but the improvement was higher in women 39 years old (OR 1.39; 95% CI 1.07–1.81 in the younger group and OR 1.60; 95% CI 1.04–2.47 in the older group). The freeze-all strategy had no impact on miscarriage rates neither in the younger (OR 0.91; 95% CI 0.55–1.51) or the older group (OR 1.43; 95% CI 0.69–2.97).
Limitations, reasons for caution: The main limitation of our study is its retrospective nature. Further, the reasons for applying a freeze-all strategy might change with the patient age, possibly affecting results.
Wider implications of the findings: Performing a freeze-all strategy seems to yield better reproductive outcomes when compared to a fresh embryo transfer. The increase in clinical pregnancy rates is higher in women 39 years or older. Clinicians should consider a freeze-all strategy in these women in order to improve their reproductive outcomes.
Trial registration number: NA

O-011 Very young women are at risk of having a high embryo aneuploidy rate

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Study question: Is the prevalence of embryo aneuploidy rate higher in very young women?
Summary answer: Women younger than 22 years old have a higher embryo aneuploidy rate, therefore they have an increased risk of producing chromosomally abnormal offspring.
What is known already: The embryo aneuploidies frequency is estimated to be high and its occurrence is related to maternal and paternal factors. The main factor is female age. It has been demonstrated that increased aneuploidy rates in oocytes and embryos are observed relative to increasing maternal age, in addition, implantation rates have been shown to decrease. Since the introduction of new PGS-techniques more data have been added to elucidate the age-factor effect on aneuploidies. Taking into the account that the average age of women that attend to an IVF clinic is increasing the embryo aneuploidy rate evaluation in very young women remains difficult.
Study design, size, duration: We performed a retrospective observational study (from January 2013 to December 2015). We analysed 1040 blastocyst from 376 comprehensive chromosome screening (CCS) cycles. In order to show the relationship between female age and aneuploidies and also to avoid the confounding effect of male factor 99 polar body chromosome analysis of young women was included in the study.
Participants/materials, setting, methods: PGS was performed to couples who attended with a previous clinical history of repetitive miscarriage, recurrent implantation failure or severe male factor. For PGSv2.0, polar body and trophoectoderm genome was amplified and aCGH performed using Agilent SurePrintG3 8 × 60 K. The association between variables and female age was evaluated by chi-square (SPSSv20.0).
Main results and the role of chance: Results from CCS were obtained in 97.2% of the biopsied embryos (1093/1125). Overall, the embryo aneuploidy rate was 40.6%. The initial analysis was to determine the prevalence of aneuploidy embryos according to the age of the woman. Ages of the women ranged from 19 to 45 years. As expected, the embryo aneuploidy rate increases with age following a fifth order exponential curve. The distribution was statistically significant ($p < 0.05$). The highest embryo aneuploidy rate was observed in 43 years women (83.3%). Within the younger women (<30) the highest embryo aneuploidy rate was observed at 21 years (52.2%). Subsequently, the data were stratified into groups (<21, 22–25, 26–30, >30). The prevalence of aneuploidy was the lowest between ages 22 and 25 (24.1%). Surprisingly, the embryo aneuploidy rate for women younger than 22 years was 40.0%. Statistical significance difference between embryo aneuploidy rate and age ranges was reported ($p < 0.05$). In order to prove this result avoiding confounding factors we analysed the aneuploidies of 99 polar bodies in young women, ranged from 19 to 31 years. To summarize, results were obtained in 93% of the biopsied oocytes. The polar body aneuploidy rate was 18.5%. No difference in the results of polar body aneuploidies and age was shown.
Limitations, reasons for caution: A higher sample size design should be used to corroborate the current findings. In addition, research into the mechanism of maternal aneuploidy and age is needed.
Wider implications of the findings: This investigation reveals that women younger than 22 years show a high embryo aneuploidy rate similar to patients aged 34 years. Moreover, to show this effect in polar body a higher number of analyses is need to reach significance difference.
Trial registration number: No trial.

O-012 Is fertility treatment an additional perinatal risk factor in women over 40 years old?

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Study question: To compare perinatal outcomes in patients aged 40 years and older who conceive by assisted reproductive technologies as compared to patient who conceive spontaneously.
Summary answer: Older women (≥40 years) who conceived *via* ART demonstrate higher rates of pregnancy complications and adverse perinatal outcomes compared with similar women who conceived spontaneously.

What is known already: Advanced maternal age is a well-established risk factor for pregnancy complications including hypertensive disorders (gestational hypertension and pre-eclampsia), pre-gestational and gestational diabetes mellitus, and cesarean section. In addition, advanced maternal age is a risk factor for poor perinatal outcome including perinatal death, preterm delivery, and low birth weight. Assisted reproductive technologies (ART) including both ovulation induction and *in vitro* fertilization (IVF), have long been shown to be independently associated with different adverse perinatal outcome. However, it is less clear whether the mode of conception further adversely affects pregnancy outcomes in women over forty.

Study design, size, duration: A retrospective population-based study comparing pregnancy perinatal outcomes in all singleton pregnancies of women aged 40 years and older delivering at the Soroka University Medical Center between 1988 and 2014 was conducted. A comparison was performed between pregnancies conceived by IVF ($n = 130$, 1.5%), ovulation induction (OI; $n = 234$, 2.6%) and those with spontaneous pregnancies (SP, $n = 8566$, 95.9%). Data was collected from the electronic database and patients charts.

Participants/materials, setting, methods: The study population included all women (>40 years of age) who conceived a singleton pregnancy by IVF or OI and delivered during the study period. The comparison group consisted of all other women (>40) who delivered during the same time period, and conceived spontaneously. Demographic, obstetrical and perinatal data were compared. Multiple regression models were constructed to define independent predictors of adverse perinatal outcome. A p value of <0.05 was considered statistically significant.

Main results and the role of chance: Women in the IVF group were significantly older as compared to OI and SP ($p < 0.001$). Gestational age at delivery was significantly lower in IVF pregnancies (37.2 ± 2.48) compared to OI (37.6 ± 2.29) and SP (38.7 ± 2.49, $p < 0.001$). Pre-eclampsia and gestational diabetes mellitus demonstrated a linear association to conception mode with highest rates in IVF pregnancies and gradually lower in OI and SP. Similarly, higher rates of preterm labor (both <37 weeks and <34 weeks)) were observed in IVF pregnancies compared to OI and SP ($p < 0.001$). Cesarean section rate was significantly higher in IVF pregnancies compared to OI and SP (74.6%, 70.1%, 26.5%, respectively, $p < 0.001$). IVF pregnancies resulted in significantly higher rates of low birth weight (<2500 gr), very low birth weight (<1500 gr), intra-uterine growth restriction, and breech presentation (all $p \leq 0.001$). No association between conception methods and perinatal mortality was observed in both the uni- and multivariate analyses. However, in a multivariate model constructed for prediction of preterm delivery (controlling for maternal age, diabetes, and pre-eclampsia) both IVF and OI demonstrated a significant independent association (OR 3.2, 2.0, respectively, $p < 0.001$). The same was found for growth restriction with an OR of 5.8 for IVF and of 2.0 for OI.

Limitations, reasons for caution: The study is a retrospective population based study with its inherited limitation.

Wider implications of the findings: An increased risk for pregnancy complications and adverse perinatal outcome in pregnancies conceived following assisted reproductive techniques was observed. Pregnancies conceived following IVF treatments are at a higher risk compared with these conceived *via* ovulation induction and spontaneous pregnancies.

Trial registration number: The study is retrospective hence registration was not required. The study was approved by the local IRB committee.

O-013 Predictive value of very low levels of serum Anti-Mã1/4llerian hormone for pregnancy rate

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Study question: Are serum Anti-Müllerian hormone (AMH) levels a predictor of pregnancy rate?

Summary answer: Serum AMH levels are not predictive of clinical pregnancy rate. Indeed, patients with very low AMH levels have slightly diminished but acceptable pregnancy rate.

What is known already: AMH is an established marker of ovarian reserve and it is strongly correlated with female age. There is no doubt about a positive correlation between serum AMH levels and ovarian response. However, there is no consensus about low AMH values to either recommend or not undergoing ovarian stimulation to infertile patients.

Study design, size, duration: Retrospective, cohort study. This study includes 5570 ICSI cycles performed between 2008 and 2014 Patients were classified in 6 groups according to the serum AMH levels: ≤0.21 ng/ml (Group-I), 0.22–0.8 ng/ml (G-II), 0.81–1.7 ng/ml (G-III), 1.71–3.2 ng/ml (G-IV), 3.21–5.39 ng/ml (G-V) and ≥5.40 ng/ml (G-VI).

Participants/materials, setting, methods: University-affiliated infertility clinic. AMH serum levels were determined in duplicate by using a commercial ELISA kit (Gen-II, Beckman). Data on age, BMI, years of infertility and AFC were collected. Cycles were performed after controlled ovarian stimulation, and for each cycle dose of gonadotropins, duration of stimulation, number of obtained follicles, estradiol serum levels the day of trigger, and clinical results after embryo transfer were recorded. All variables were statistically analyzed by using the SPSS software.

Main results and the role of chance: Strong significant differences in female age (from 39.2 ± 0.3 in G-I to 35.7 ± 0.4 in G-VI), AFC (from 1.93 ± 0.2 in G-I to 6.35 ± 1.1 in G-VI), FSH dose (from 1606 ± 95 in G-I to 1392 ± 53 in G-VI), oocytes retrieved (from 2.9 ± 0.3 in G-I to 14.0 ± 2.0 in G-VI) and mature oocytes (from 2.9 ± 0.3 in G-I to 14.0 ± 2.0 in G-VI) were found between groups. By contrast, the years of sterility, BMI and days of stimulation were similar in all the groups.

Regarding clinical results, neither implantation rates (28.9% in G-I, 25% in G-II, 29% in G-III, 28.8% in G-IV, 30.5% in G-V and 35.3% in G-VI) nor abortion rate (27.9%, 26.8%, 31%, 28.8%, 25.7% and 22.1%, respectively) showed significant differences between groups. Although clinical pregnancy rates were significantly different in the groups, the very low serum AMH level group retained a significant chance of reproductive outcome (45.3%, 48.1%, 47.4%, 45.2%, 48.0% and 54.1%, respectively). However, ROC curve showed no predictive value of AMH related to clinical pregnancy.

Limitations, reasons for caution: Due to the retrospective design, we cannot rule out patients with very low AMH levels that decided not to do ovulation stimulation. Thus, prospective validation is required.

Wider implications of the findings: Serum AMH levels are closely related to female age and it is a very robust marker of ovarian reserve and ovarian response to gonadotropins. By contrast, it has no predictive value for clinical pregnancy, and even patients with very low serum AMH levels still have a reasonable reproductive outcome.

Trial registration number: Not applicable

O-014 Perinatal outcomes following oocyte donation versus autologous IVF: analysis of 99,111 singleton live births

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Study question: Does oocyte donation influence perinatal outcomes of preterm birth (PTB) and low birth weight (LBW) compared to autologous IVF treatment.

Summary answer: There was an increased risk of adverse perinatal outcomes of PTB and LBW following oocyte donation compared to autologous IVF treatment.

What is known already: There has been an increasing burden of poor ovarian response following IVF attributed to women delaying childbearing and consequently women of advanced age seeking IVF. However, little information is available regarding maternal or infant outcomes following oocyte donation. There is a higher risk of pregnancy complications following assisted reproductive treatments (ART) compared to spontaneously conceived pregnancies attributed to the underlying infertility itself or embryo specific epigenetic modifications due to the *in vitro* fertilisation techniques. It is a matter of interest if oocyte donation influences obstetric outcomes and whether use of donor oocytes affects perinatal outcomes compared to pregnancies following autologous IVF.

Study design, size, duration: Anonymous data were obtained from the Human Fertilization and Embryology Authority (HFEA), the statutory regulator of

assisted reproduction treatment (ART) in the UK. The HFEA has collected data prospectively on all ART performed in the UK since 1991. Data from 1991 to 2011 involving a total of 524,489 fresh oocyte donor and autologous IVF cycles resulting in 99,111 singleton live births were analysed.
Participants/materials, setting, methods: Data from all women who underwent either fresh donor oocyte or autologous IVF cycles during the study period were analysed to compare perinatal outcomes of PTB, early PTB, LBW and very LBW following singleton live births. Logistic regression analysis was performed adjusting for female age category, year of treatment, number of previous IVF cycles, type of infertility, causes of infertility, number of oocytes retrieved and initial multiple vs. singleton gestation leading to singleton live birth.
Main results and the role of chance: The unadjusted odds of PTB (OR 2.24, 95% CI 1.71 to 2.93), early PTB (OR 1.97, 95% CI 1.13 to 3.44), LBW (OR 1.98, 95% CI 1.50 to 2.63) and very LBW (OR 1.60, 95% CI 1.15 to 3.0) were significantly higher with donor oocyte compared to autologous IVF. There was a significant increase in the risk of adverse perinatal outcomes of PTB and LBW following oocyte donation after adjusting for potential confounders such as female age category, treatment period, previous IVF cycles, type of infertility, cause of infertility, number of oocytes retrieved and initial multiple vs. singleton pregnancy leading to singleton live birth: PTB adjusted odds ratio (aOR) 2.29, 95% CI 1.73 to 3.03 and LBW aOR 2.0, 95% CI 1.50 to 2.69. The aOR for early PTB was 1.63, 95% CI 0.91 to 2.91 and aOR for very LBW was 1.29, 95% CI 0.67 to 2.49.
Limitations, reasons for caution: Although the analysis was adjusted for a number of important confounders, the dataset had no information on confounders such as smoking, body mass index (BMI) and the medical history of women during pregnancy to allow adjustment.
Wider implications of the findings: Analysis of this large dataset suggests that there is increased risk of PTB and LBW following oocyte donation. This information is useful to counsel women and for clinicians managing pregnancies resulting from oocyte donation IVF.
Trial registration number: not applicable

SELECTED ORAL COMMUNICATIONS

SESSION 04: SPERM SELECTION AND PREPARATION FOR ART

Monday 04 July 2016 **Hall 5 A** **10:00–11:30**

O-015 Prediction model for live birth in intracytoplasmic sperm injection using testicular extracted sperm

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Study question: Which parameters have a predictive value for live birth in couples undergoing intracytoplasmic sperm injection using testicular extracted sperm (TESE-ICSI)?
Summary answer: Female age, a first or subsequent cycle, male luteinising hormone, male testosterone, sperm motility and the initial aetiology of azoospermia were relevant predicting parameters.
What is known already: In reproductive medicine prediction models are used to predict treatment success, but no prediction model currently exists for live birth after TESE-ICSI.
Study design, size, duration: A retrospective cohort study between 2007 and 2015 in two academic hospitals including 1559 TESE-ICSI cycles. The prediction model was developed using data from one centre and validation was performed with data from the other centre.
Participants/materials, setting, methods: All couples undergoing TESE-ICSI treatment for the first time. In the development set, we included 526 couples undergoing 1006 TESE-ICSI cycles. We included 289 couples undergoing 553 TESE-ICSI cycles in the validation set. Multivariable logistic regression models were constructed in a stepwise fashion ($p < 0.2$ for entry). The external validation was based on discrimination and calibration.
Main results and the role of chance: A total of 224 couples (22.3%) with a live birth in the development set were included. The occurrence of a live birth was associated with lower female age, first TESE-ICSI cycle, lower male LH, higher male testosterone, the use of motile spermatozoa for ICSI and having obstructive azoospermia as an initial aetiology of azoospermia. The area under the receiver operating characteristic (ROC) curve was 0.62. From validation data, the model had moderate discriminative capacity (c-statistic 0.67, 95% confidence interval: 0.62–0.72) but calibrated well, with a range from 0.06 to 0.56 in calculated probabilities.
Limitations, reasons for caution: Data about the motility of spermatozoa during TESE is lacking. Therefore, we used data about the motility of the spermatozoa used for ICSI after freeze–thawing. In counselling before start of ICSI treatment, those two options (motile spermatozoa available for ICSI or not) should be taken into account.
Wider implications of the findings: This model enables the differentiation between couples with low and couples with high chance to reach a live birth using TESE-ICSI. As such it can help counselling patients and in clinical decision making.
Trial registration number: Not applicable.

O-016 Prediction model for obtaining spermatozoa with TESE in men with non-obstructive azoospermia

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Study question: Can we build a prediction model for successful testicular sperm extraction in men with non-obstructive azoospermia and will the model be accurate after external validation?
Summary answer: Our prediction model including six variables was able to predict the chance of obtaining spermatozoa with TESE.
What is known already: Only a few models have been developed that predict the chance of obtaining spermatozoa with TESE, and none of them have been validated externally.
Study design, size, duration: We performed a prospective nationwide cohort study. Data of 1371 TESE procedures were collected between June 2007 and June 2015 in the fertility centres of the Radboud university medical center in Nijmegen and the Academic Medical Center in Amsterdam.
Participants/materials, setting, methods: All men with NOA undergoing their first TESE procedure were included. Primary endpoint was the presence of spermatozoa in the testicular biopsies. We constructed a model for the prediction of successful sperm retrieval, using univariable and multivariable binary logistic regression analysis and the dataset from one centre. This model was then validated using the dataset from the other centre. The area under receiver operating characteristic curve was calculated and model calibration was assessed.
Main results and the role of chance: There were 599 (43.7%) successful sperm retrievals after a first TESE procedure. The prediction model, build after multivariable logistic regression analysis, demonstrated that higher male age, higher levels of serum testosterone and lower levels of FSH and LH were predictive for successful sperm retrieval. Diagnosis of idiopathic NOA and an AZF-c deletion were predictive for unsuccessful sperm retrieval. The area under the curve was 0.69 (95% CI: 0.66 to 0.72). The difference between the mean observed chance and the mean predicted chance was less than 2.0% in all groups, indicating good calibration. In external validation, the model had moderate discriminative capacity (area under the curve 0.65, 95% CI: 0.62 to 0.72) and moderate calibration, the predicted probability never differed more than 9.2% of the mean observed probability.
Limitations, reasons for caution: This model is limited to use prior to the first TESE procedure only.

Wider implications of the findings: This model can help in clinical decision making in men with NOA by reliably predicting the chance of obtaining spermatozoa with TESE.
Trial registration number: Not applicable

O-017 Meaning of DNA fragmentation in relation to the sperm source and ART outcome

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Study question: We question whether sperm chromatin integrity differs among spermatozoa isolated from different sections of the male genital tract and how it affects reproductive outcome.
Summary answer: Progression through the male genital tract increases chances for oxidative aggression and consequent sperm chromatin fragmentation (SCF), this may be obviated by utilizing testicular spermatozoa.
What is known already: During the later stages of spermiogenesis DNA breakage is physiologically induced to allow tight chromatin compaction. While most spermatozoa undergo DNA repair, additional reactive oxygen species (ROS) are the main cause for DNA injury. The buffering capacity of seminal antioxidants is the only agent protecting the DNA integrity of spermatozoa in the ejaculate. Therefore, retrieving spermatozoa from the epididymis or testis may bypass such an insult on the chromatin.
Study design, size, duration: Over 27 months, men with extremely high SCF in their ejaculates ($n = 64$) underwent surgical sampling, often bilateral, from vas deferens, epididymis, and testis. SCF was assessed by terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) and clinical outcome was recorded for each sperm source for men undergoing ICSI.
Participants/materials, setting, methods: Ejaculates processed in standard fashion were assessed for SCF by TUNEL. Surgical samples were minced and smeared for SCF evaluation and were cryopreserved for later use with ICSI. DNA fragmentation was measured by TUNEL on specimens isolated from all sites. TUNEL was assessed utilizing a commercial kit (In Situ Cell Death Detection Kit, Roche). At least 500 spermatozoa were evaluated per site under fluorescent microscopy with an adopted threshold of 15%.
Main results and the role of chance: Of the original 64 patients, 51 were treated by ART with an average SCF of 31.0 ± 19% (range 26.0–96.0). In 9 men aspiration of the vas deferens resulted in 16.7 ± 8% SCF (range 5.8–30.0) while in 32 men epididymal sampling yielded 17.3 ± 8% SCF (range 7.0–34.8) and in 64 the SCF on testicular spermatozoa was 12.3 ± 6% (range 2.0–27.0). The SCF progressively decreased as TUNEL was performed proximally from the ejaculate toward the vas deferens ($P = 0.05$), the epididymis ($P = 0.01$), and testis ($P = 0.01$). ART outcome with ICSI utilizing these surgical sources yielded a clinical pregnancy of 26.7%, while with the ejaculated counterpart only 14.6%. Based on these preliminary findings a subgroup of patients ($n = 19$), with SCF of 40.1 ± 18 bypassed the prerequisite cycle with ejaculated spermatozoa. By opting to directly undergo TESE with ICSI a clinical pregnancy rate of 29.0% per cycle was achieved that translated to 55.6% per couple treated.
Limitations, reasons for caution: Patients need to be informed of risks regarding surgery, anesthesia, and be aware that even with surgical spermatozoa a pregnancy may not occur. Thus, engaging counseling should be conducted since many of these men have spermatozoa in their ejaculate. These data are still preliminary and require an evidence-based consensus.
Wider implications of the findings: DNA integrity assessment on the spermatozoa isolated at different levels of the male genital tract evidenced that oxidative stressors progressively compromise DNA integrity toward the ejaculate. Couples unable to achieve a pregnancy using ejaculated spermatozoa with compromised DNA may benefit from undergoing testicular retrieval for diagnostic and therapeutic purposes.
Trial registration number: N/A

O-018 Parameters predicting successful sperm retrieval in men with non-obstructive azospermia undergoing primary and repeated microdissection testicular sperm extraction

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Study question: What are the predictive parameters for the microdissection testicular sperm extraction (m-TESE) outcome in men with non-obstructive azoospermia (NOA) undergoing primary and repeated m-TESE
Summary answer: Prior unsuccessful m-TESE and low testicular volume were the significant negative predictors in repeated m-TESE cases but there were none for primary cases.
What is known already: M-TESE is the recommended method for sperm retrieval and the sperm retrieval rate is around 50% in primary m-TESE cases. Chromosome Y microdeletion is the only prognostic parameter useful in predicting m-TESE outcome but we still need other predictive factors. There are a limited number of studies which have evaluated preoperative parameters predicting m-TESE success. One of these studies concluded that history of Klinefelter Syndrome and orchiopexy are the most significant predictors, whereas the other suggested inhibin-B levels to be predictive of m-TESE outcome.
Study design, size, duration: A total of 518 men with NOA undergoing primary ($n = 225$) and repeated ($n = 293$) m-TESE procedures between October 2011 and September 2015 were evaluated retrospectively. Serum hormone levels (gonadotropins, total testosterone, estradiol and Inhibin-B), genetic investigations as well as detailed physical examinations were done. If the patients had hyper/hypogonadotropic hypogonadism, hyperprolactinemia, Testosterone/E2 ratio less than 0.10 in addition to having previous unsuccessful m-TESE operation, medical treatment was offerred for 5–6 months.
Participants/materials, setting, methods: Parameters such as age, total testicular volume (TTV), hormone levels before and after treatment, and previous m-TESE results were compared between men with successful and unsuccessful m-TESE within the primary and repeated m-TESE groups. A multivariate logistic regression analysis was used to determine the independent parameters correlated with m-TESE outcome for each group.
Main results and the role of chance: Overall sperm retrieval rate (SRR) was 44.8%, of which the ratio was 43.6% in primary and 45.7% in repeated m-TeSe groups. Age (33.6 vs. 36.2) and E2 levels (29.2 vs. 34.2) were significantly different between primary and repeated m-TESE groups. Age was positively and E2 levels were negatively correlated with the increasing number of prior m-TESE. All of the parameters were similar between patients with successful and unsuccessful m-TESE in primary micro-TESE group, whereas patients in repeated m-TESE group with successful outcome were older, had significantly higher TTV and 84.7% of the patients had prior successful m-TESE ($p < 0.001$) (Table1). After multivariate logistic regression analysis, higher TTV and prior successful m-TESE were correlated with successful m-TESE outcome in repeated m-TESE group, but age did not. However, none of the parameters were predictive of the successful m-TESE outcome in primary m-TESE group.
Limitations, reasons for caution: We did not find Inhibin B as a significant predictor of m-TESE outcome in contrast to a prior study. This might be due to the limited number of serum Inhibin B measurements in our study population. Therefore, importance of this hormone needs further evaluation especially for primary m-TESE cases.
Wider implications of the findings: This is the first study evaluating parameters predictive of TESE outcome in both primary and repeated m-TESE cases. According to our results patients with prior failed m-TESE and low TTV showing testicular atrophy due to testicular tissue damage should be councelled regarding the decreased chances of success after repeated procedures.
Trial registration number: None

O-019 Clinical freeze preservation of whole human testicular tissue: the practicality of pre-freeze *in vitro* culture (IVC) at 30Â°C to insure good post-thaw sperm motility

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Study question: Can testicular tissue be processed in a simplistic and effective manner that promotes progressive sperm motility to enhance and optimize overt post-thaw sperm viability?

Summary answer: The cryopreservation of small testicular tubular masses following extended IVC (48–96 h) effectively stimulated pre-freeze and post-thaw sperm motility while reducing technician processing time and labor.
What is known already: Frozen-thawed testicular sperm has been effectively used for two decades, resulting in live births rates similar to fresh testis sperm. It has been shown that the use of intermediate temperature conditions (30°C ± 2°C) can promote and extend the motility potential of immature testicular sperm, whereas 21°C IVC conditions fail to optimize motility potential (% and progression) and 37°C maximizes metabolic activity which reduces longevity. IVC itself, as well as effective cryopreservation, reduces the urgency to synchronize surgical procedures (i.e., testicular biopsies and oocyte retrievals). Meanwhile, the ease of handling whole tissue masses minimizes the time and labor of processing.
Study design, size, duration: Over a 24 month interval between 2014 and 2015, 40 adult men (24–62 years old) were scheduled to have testicular tissue frozen for possible future ICSI use. A retrospective analysis was performed to demonstrate the efficacy of our whole tissue freeze preservation/IVC methodology for cryobanking purposes. We assessed sperm motility patterns for up to 1 week and compared pre-freeze and post-thaw total and progressive sperm motility patterns, contrasting differences by ANOVA ($p < 0.05$).
Participants/materials, setting, methods: Surgically recovered testicular tissue was transported to the lab in sperm wash medium under insulated ambient conditions. Needle dissected, small tubular masses were placed into individual cryovials (0.5 ml medium), or a test piece was shred and cultured under oil in a heated Styrofoam box (30°C ± 1°C), along with the vials. Upon daily assessments of motility (I = twitching to IV = rapid progression), when greater than 10–20% progressive motility was attained, samples were frozen. Test thaws were performed/evaluated at +3 h.
Main results and the role of chance: Testicular tissue freeze preservation services were provided to men scheduled for either a Vasovasostomy surgery ($n = 32$, 84%) or to assist NOA/Anejaculatory Cancer patients ($n = 8$). Only two of the latter NOA patients failed to yield sperm, thus 95% of the clients had tissue cryopreserved. Total and progressive motility significantly elevated by +48 h IVC (46.8% and 12.4%) compared to 26.4% and 5.2% at +3 h and 32.5% and 5% at +24 h, respectively. Total motility peaked at +96 h (52.1%), while progression elevated ($p < 0.05$) to 20.3% and 24.5% by +96 and 120 h, respectively, and remained high at +168 h (20+%). Mean pre-freeze total and progressive sperm motilities declined ($p < 0.05$) from 44.4% ± 2.0% SE and 13.3% to 32.1% ± 2.2 and 6.7%, respectively, post-thaw. The reduced motility recovery rate of both total (73%) of progressive (50%) movements, are generally lessened by additional overnight IVC considering the median pre-freeze IVC interval was +72 h. The motility patterns of pre-freeze/equilibrated (3 h, 37°C), post-thaw samples were: I = 18.7%/17%; II = 12.8%/8.4%; 9.6%/5.1%; and IV = 3.7%/1.6%, respectively. Overall, excellent conservation of sperm viability was achieved, with minimal pre-freeze processing, by cryopreserving whole testicular tissue pieces in a 13.4% glycerol solution and five equal mixed dilutions.
Limitations, reasons for caution: Testicular freeze-preservation of Vasovasotomy patients is essentially an insurance policy to prevent a possible second surgery in case the reversal fails to be effective and future IVF treatment is required. Any concerns regarding IVC- or cryo-related reactive oxidative stress DNA fragmentation have been proven negligible (Schiewe et al., 2016).
Wider implications of the findings: Whole testicular tissue freezing following IVC promoting motility enhancement has proven be a highly effective, while minimizing excessive and laborious processing procedures. The availability of overtly motile sperm, simplifies valuable Embryology time to isolate viable sperm to ICSI oocytes in a timely manner.
Trial registration number: None

O-020 Choosing the appropriate insemination method according to sperm DNA fragmentation level

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Study question: We question if sperm chromatin fragmentation (SCF) can guide toward the appropriate ART method in couples with idiopathic infertility and history of poor IUI outcome.
Summary answer: This study provides a DNA fragmentation-based algorithm that allows appropriate allocation of resources and guides patients towards the appropriate infertility treatment.
What is known already: During the later stages of spermiogenesis DNA breakages are physiologically induced to allow tight chromatin compaction and only those spermatozoa with repaired chromatin reach the ejaculate. Throughout the male genital tract oxygen-free radicals, mostly from decaying spermatozoa and other cells, are the main cause of DNA damage and responsible for the impaired ART outcome. Therefore, in men with adequate semen parameters but poor reproductive outcome, a SCF assessment may guide towards the most effective ART treatment.
Study design, size, duration: Over a 27-month period, couples were allocated to the appropriate ART treatment according to the level of SCF. The clinical pregnancy rate was evaluated according to SCF cohort and in the relation to each insemination method.
Participants/materials, setting, methods: Infertile couples ($n = 551$) underwent standard semen analysis according to WHO criteria (2010) and resulted in adequate semen parameters for IUI and ART. Couples that failed IUI were then screened for chromatin fragmentation by TUNEL and/or SCSA® on their ejaculates. In-house TUNEL assessment evaluates 500 spermatozoa under fluorescent microscopy and patients were only deemed abnormal when sperm DFI reached ≥15%. SCSA® assessment was performed by an external laboratory and analyzes 5000 spermatozoa (abnormal >25%).
Main results and the role of chance: A total of 551 couples underwent 1363 cycles and included women with an average age of 37.7 ± 4 years and men with a mean age of 39.8 ± 5 years. The overall average sperm concentration was 50.0 ± 29 million with a motility of 49.9 ± 14% and morphology of 3.1 ± 2%. Based on the control, we expect an intrauterine insemination (IUI) clinical pregnancy rate of 17.9%, but the study cohort evidenced a clinical pregnancy rate of just 3.4%. The patients from this cohort presented a TUNEL of 26.1 ± 18% and SCSA DFI of 39.5 ± 26%. Men with normal SCF were subsequently treated by *in vitro* insemination and reported a pregnancy rate of 22.1%. Once we controlled for an eventual confounding female factor (female age ≤35 years), a remarkably higher pregnancy rate of 36.8% ($P < 0.001$) was reached. On the other hand, couples with abnormal DFI were treated exclusively by ICSI also yielding a higher pregnancy rate at 21.7%, and 28.9% with females 35 years old ($P < 0.001$). For those patients that failed ICSI with ejaculated spermatozoa, we offered a testicular sampling. In 38 couples that consented, the SCF of testicular spermatozoa was 12.3 ± 6%, remarkably lower than 39% SCF in the ejaculate, and a pregnancy rate of 26.7% ($P < 0.001$).
Limitations, reasons for caution: Patients need to be informed of risks regarding surgery, anesthesia, and the possibility that even with TESE a pregnancy may not occur. Thus, engaging counseling should be conducted since these men have spermatozoa in their ejaculate. These data are still preliminary and a clinical consensus has not been reached.
Wider implications of the findings: IVF is successful in men with intact sperm chromatin. When sperm SCF is compromised in the ejaculate, ICSI is the most suitable insemination method. In men with high DNA fragmentation in their ejaculate and pursuant pregnancy failure, surgical sampling yields spermatozoa with lower SCF and higher changes of pregnancy.
Trial registration number: N/A

SELECTED ORAL COMMUNICATIONS

SESSION 05: ADVANCES IN UNDERSTANDING OF ENDOMETRIOSIS AND ENDOMETRIAL BIOLOGY

Monday 04 July 2016 **Hall 3 AB** **10:00–11:30**

O-021 Genome-wide DNA methylation and mRNA expression profiling in eutopic endometrium, disease tissue and fat: implications for endometriosis research

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Study question: What implications does variability in transcriptomic/epigenomic profiles in whole tissues relevant to endometriosis have for "omics" study designs?
Summary answer: Inter-individual variation is greater than intra-tissue and technical variability in all three tissues, and we identify menstrual phase as the strongest covariate.
What is known already: Of the nine genetic loci associated with endometriosis, most are intergenic with unclear functional roles. Integrated analysis of genomic with transcriptomic and epigenomic data can help identify biological-pathways perturbed by these genetic variants. Studies in other disease areas have shown that these investigations need to be conducted in tissue relevant to disease. For endometriosis this is endometrium, which is not included in large profiling initiatives such as GTex and NIH-Epigenome-Roadmap. However, the utility of tissue-based profiling for endometrium requires further exploring, as it is a heterogeneous tissue composed mainly of glandular epithelial and stromal cells subject to cyclic endocrine influences.
Study design, size, duration: The prospective ENDOX study recruits women undergoing laparoscopy for symptoms suggestive of endometriosis or tubal sterilisation. Twenty-four women (8 endometrioma cases; 8 with peritoneal endometriosis; 8 controls) provided 48 tissue samples using WERF EPHect protocols (16 eutopic and 16 ectopic endometrium, 16 subcutaneous adipose tissue).
Participants/materials, setting, methods: Subjects were not using hormones in the previous 3 months and had regular cycles; controls were frequency-matched to cases on menstrual phase. Tissues were split for DNA vs. RNA extraction. In each extraction arm, the 32 eutopic and ectopic endometrium and 8/16 adipose tissue samples were split prior to extraction; 10% of samples were analysed in duplicate. DNA and RNA samples (n = 96 each) were analysed using Illumina 450Kmethylation and H12 expression arrays, respectively.
Main results and the role of chance: Hierarchical-cluster-analysis showed distinct methylation profiles per tissue type; peritoneal-disease-tissue and endometrioma samples also clustered distinctly, suggesting different methylation signatures. Intra-tissue variability was investigated through analysis of split samples of the same tissue, while technical variation was assessed through replicates. The principal-component-analysis demonstrated that the inter-individual (biological and environmental) variation was greater than intra-tissue (cellular heterogeneity) and technical (experimental) variation in all three tissues. This was supported by variance-component-analysis of the top 10% most variable methylation probes, which showed that inter-individual variance explained the majority of the overall methylation variance. Menstrual phase was the covariate most significantly correlated with methylation patterns in eutopic endometrium and fat, but not in ectopic endometrium. Smoking showed a strong correlation with methylation profiles of fat and ectopic disease tissue. Analysis of the eutopic endometrium gene expression data supported the findings of greater inter-individual variation compared with intra-tissue and technical variation, while menstrual phase was also the most important covariate. Power calculations showed that to have 80% power for detection of differentially methylated/expressed variants with effect size >0.4, minimum 500 tissue samples are required. As an outset, differential expression analysis adjusted by menstrual phase between cases and controls identified 3 loci with fold change expression >4.
Limitations, reasons for caution: This a pilot study performed to test parameters affecting tissue profiling variability and their implication for larger study designs. Due to the size of the study, the effects of more subtle covariates would not have been detected. Any significant differential expression/methylation result need to be replicated in a larger study.
Wider implications of the findings: This is the first study investigated tissue expression and methylation profiling variability in tissues relevant to endometriosis. These results enable appropriate study design of future large-scale collaborative studies.
Trial registration number: N/A

O-022 Co-expression of the estrogen receptor beta splice variant isoform 5 (ERÃŸ5) with estrogen receptor alpha selectively augments estrogen responsiveness of endometrial epithelial cells

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Study question: Does the ERβ5 variant protein play any role in endometrial function or malfunction?
Summary answer: ERβ5, an isoform which lacks an intact ligand binding pocket may alter the response of epithelial cells to oestrogens by forming heterodimers with ERα.
What is known already: Oestrogens play an essential role in regulation of endometrial function during the menstrual cycle. Their biological effects are classically mediated *via* oestrogen receptors that act as ligand-activated transcription factors. Two oestrogen receptors have been identified; oestrogen receptor alpha (ERα) and beta (ERβ). Multiple isoforms of the ERβ protein have been described: mRNAs encoding full length (ERβ1) and truncated variants (ERβ2 and ERβ5) are present in normal endometrium but protein expression has not been described. ERβ5 protein is highly expressed in epithelial cells in endometrial adenocarcinomas. ERβ5 cannot bind E2; its role in mediating cell responses to E2 is poorly understood.
Study design, size, duration: Endometrial biopsies collected from women with written informed consent and local research ethics committee approval (LREC/07/S1103/29) were processed for immunohistochemistry. ERα-positive Ishikawa cells were used to investigate the impact of with ERβ1 or ER5 on E2 responses. An ERβ5 receptor protein with a yellow fluorescent protein "tag" (ERβ5-YFP) was used in fluorescence recovery after photobleaching (FRAP) experiments. Transcription assays were conducted in cells cotransfected luciferase cDNA under the control of an oestrogen response element (ERE-luc).
Participants/materials, setting, methods: Immunohistochemistry was performed on NBF-fixed paraffin-embedded endometrial tissue. Ishikawa cells were used as a model of endometrial epithelial cells. FRAP was conducted using a Zeiss LSM 510 laser scanning confocal microscope with images captured at 3 s intervals after bleaching. Cells used in reporter assays were engineered to express different ratios of ERα and ERβ5. Reporter gene activity was measured after treatment of cells with vehicle control (ethanol) or E2 10^{-8} M for 24 h.
Main results and the role of chance: ER5 was immunolocalised to epithelial and stromal cells; region/stage-dependent variation in epithelial cells was observed. In cells transfected with YFP-ERβ5, treatment with E2 identified two populations of cells: a) cells in which treatment with E2 had no impact, b) cells in which E2 treatment affected mobility of ER protein. In these cells, treatment with E2 resulted in rapid redistribution of the protein, reduced intra-nuclear mobility and a redistribution of the receptor into a punctate pattern (70% of cells 30 min after addition of E2). As ERβ5 lacks an intact ligand binding pocket and we have previously shown mobility was unchanged in cells which do not express full length ER proteins, we investigated whether punctate redistribution was a result of ERβ5 forming heterodimers with ERα. When the ratio of ERα and ERβ5 was altered in Ishikawa cells, the response of an ERE-driven reporter gene to E2 was significantly increased in cells with an increased ratio of ERβ5:ERα. The increase in E2-dependent reporter gene activation was abrogated by the antiestrogen Raloxifene. Responses to the ERα-selective agonist PPT mimicked those of E2. In contrast cotransfection with ERβ1 reduced E2 dependent reporter gene activity.
Limitations, reasons for caution: Immunohistochemistry on fixed tissue sections only reflects receptor location and abundance at a single point in time. Ishikawa cells that are commonly used as a model cell for endometrial epithelium were originally established from an endometrial adenocarcinoma.
Wider implications of the findings: ER5 is expressed in both normal endometrium and also in endometrial and other cancers: these novel data suggest we should re-evaluate its role in modulating estrogen-responsiveness in cells where it is coexpressed with ERα. Our results have important implications in understanding the regulation of proliferation in gynecological health and disease.
Trial registration number: n/a

O-023 Regulation of angiogenesis-related prostaglandin F2α-induced VEGF and CXCL-8 factors in endometriosis

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Study question: How the activation or blockade of the prostaglandin (PG) $F_{2\alpha}$ receptor (FP) might stimulate angiogenesis in ectopic endometrial implantation sites?
Summary answer: $PGF_{2\alpha}$ enhances differential expression of VEGF and CXCL-8 through two variants of FP (FP_A and FP_B) which potentiates angiogenesis by endothelial proliferation and migration.
What is known already: Active angiogenesis and proliferation processes are required for ectopic endometrial tissue growth during endometriosis. Our previous studies showed that eutopic and ectopic endometrium from women with endometriosis exhibit higher expression of key enzymes involved in the $PGF_{2\alpha}$ biosynthetic pathway (e.g., cyclooxygenase-2 (COX-2), aldoketoreductase-C3...), as well as a high expression of PGF_2 receptor (e.g., FP), in endometriotic lesions. It has also been shown that the interaction between $PGF_{2\alpha}$ and its receptor in human endometrial pathologies induces angiogenesis (e.g., adenocarcinoma).
Study design, size, duration: The production of angiogenic factors (VEGF and CXCL-8), with disruption of $PGF_{2\alpha}$ signaling pathways using, inhibitors of FP signaling: COX-2, phospholipase C, protein kinase C or Ca^{2+}chelator, was assessed by RT-qPCR, WB, and ELISA (n = 11). Angiogenesis, proliferation and migration evaluations (n = 3), following exposure to conditioned media of transfected cells either with FP_A or FP_B, following $PGF_{2\alpha}$ treatment using Matrigel® angiogenesis network and scratch wound tests were assessed by microscopy.
Participants/materials, setting, methods: The study was conducted at the CHUQ and in an inflammatory research laboratory. Primary cultures of eutopic stromal cells of control women (n = 7) and matched eutopic and ectopic stromal cells of women with endometriosis (n = 11) were exposed to different concentrations of $PGF_{2\alpha}$. Endometriosis was thus categorized as early (n = 6) or late (n = 5) stages. Of the afflicted patients, 4 were in the follicular phase and 7 in the luteal phase at the moment of sample collection.
Main results and the role of chance: $PGF_{2\alpha}$ stimulated CXCL-8 ($p < 0.05$) and VEGF ($p < 0.05$) protein secretion in endometriotic ectopic stromal cells in the proliferative and secretory phases, without noticeable changes in eutopic endometrial stromal cells of women with or without endometriosis. Cell exposure to $PGF_{2\alpha}$ analog confirmed these previous observations. After 24 h of stimulation, FP antagonism led to an inhibition of the $PGF_{2\alpha}$-induced VEGF ($p < 0.05$) and CXCL-8 ($p < 0.01$) protein secretion, thereby indicating a specific effect of $PGF_{2\alpha}$. Meanwhile, $PGF_{2\alpha}$ increased significantly COX-2 expression while its other enzymes showed no difference. The COX-2 inhibitor NS398 decreased both VEGF and CXCL-8 secretion. Furthermore, in transfected cells either with FP_A or FP_B alone, COX-2 and phospholipase C inhibition suppressed $PGF_{2\alpha}$-induced CXCL-8 secretion ($p < 0.01$ and 0.05, respectively) through FP_A, while only protein kinase C and Ca^{2+} production were involved in VEGF ($p < 0.001$ and 0.001, respectively) through FP_B. Thus, both transcripts, FP_A and FP_B, are required for the $PGF_{2\alpha}$-induced stimulation of VEGF and CXCL-8 production. Finally, $PGF_{2\alpha}$ enhanced angiogenesis through endothelial tubal formation ($p < 0.05$) and proliferation ($p < 0.05$) processes in response to FP_A cells supernatant.
Limitations, reasons for caution: We carried out this study in isolated peritoneal ectopic stromal cells, which doesn't include preservation of histotypic relationships among other endometrial cells.
Wider implications of the findings: These results show for the first time that $PGF_{2\alpha}$ exerts direct and indirect angiogenic effects *via* FP signaling pathways by interacting with ectopic stromal cells and by inducing the secretion of major angiogenic factors. This study provides evidence for a possible new mechanism underlying endometriosis development and pathophysiology.
Trial registration number: Not Applicable

O-024 Seminal plasma mediates metaplasia in endometriosis, *via* transcriptional repressors (SNAIL and ZEB)

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Study question: Can seminal plasma (SP) induce metaplasia in endometriosis (EM)?
Summary answer: SP induces a time dependent metaplasia in endometriotic cells, characterized by a rapid and transient increase in SNAIL1/2 and ZEB2 expression.
What is known already: SP, is rich in growth factors and hormones (e.g., TGFβ1 and estrogen) known to induce tissue metaplasia. Metaplasia may promote the development of EM lesions, and may be a mechanism through which abundant metaplastic myofibroblasts (expressing alpha smooth muscle actin (ASMA)) predominate in the stroma of EM lesions. **Study design, size, duration:** Semen samples were obtained from normozoospermic men (n = 14) as assessed according to WHO criteria 2010. After liquefaction, samples were centrifuged at 3000 rpm for 10 min, and the supernatant was stored at −20C. After thawing at room temperature, SP was pooled and filtered in 0.2 µm mesh and used for *in vitro* experiments in a 1:10 dilution.
Participants/materials, setting, methods: 12Z (endometriotic epithelial) and St-T1b (endometriotic stromal) cell lines (components of EM) were basically characterized *in vitro* by Immunofluorescence, for cytokeratin, vimentin and ASMA.

The effect of 2 h and 6 h incubation with SP 10% on both cell lines was studied with quantitative real-time PCR in comparison to SP-free culture medium in regard to the mRNA expression of metaplasia markers (E-cadherin, ASMA) and metaplasia mediators (SNAIL 1/2, ZEB2, and TWIST). **Main results and the role of chance:** Immunofluorescence characterization: Vimentin was expressed both in 12Z and St-T1b, while cytokeratin was only expressed by 12Z. Very few single ASMA expressing cells were observed in St-T1b, while they were absent in 12Z.

Metaplasia markers: Incubation of 12Z with SP 10% upregulated ASMA expression, but downregulated E-cadherin expression, both pointing to metaplasia. Both effects were observed after 2 h (early effect) and more pronounced after 6 h (late effect) incubation. In St-T1b, the same findings were observed for ASMA, however, in contrast to epithelial 12Z cells; an upregulation of E-cadherin was noticed. In contrary, N-cadherin was down regulated in both cell lines following SP incubation.

Metaplasia mediators: Incubation with SP 10% induced a rapid upregulation of SNAIL1/2 (as early as 2 h incubation), in addition to a more delayed upregulation of ZEB2 expression (as late as 6 h incubation). In contrast, TWIST expression was down regulated in both cell lines. This might suggest a SNAIL-mediated early metaplasia and a ZEB2-mediated late metaplasia, whereas the expression of TWIST does not follow conventional patterns.
Limitations, reasons for caution: Studying metaplasia markers and factors on the level of protein expression as well as blocking metaplasia-inducing growth factors in SP will further elucidate the precise role of SP on the development of EM.
Wider implications of the findings: Other possible effects of SP (being an inducer of metaplasia) on endometriotic cell invasion, migration and cellular viability should be studied. This will help to understand the effect of SP coming in contact with the endometrium or with vaginal EM lesions (during intercourse).
Trial registration number: Irrelevant

O-025 Detection of cancer stem cell phenotype within endometrial mesenchymal stem cells of endometriosis patients

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Study question: Endometriosis Associated Cancer (EAC) – can we detect cancer stem cell markers from stem cells in endometrium or endometriotic cyst within endometriosis patients?
Summary answer: We identify a subpopulation of endometrial mesenchymal stem cells (MSC) expressing Cancer Stem cells (CSC) markers in endometriosis patients.
What is known already: EAC is a rare but possible molecular event occurring among 0.7–2.5% of endometriosis patients. Several epidemiological multicentre studies on endometriosis patients showed an increased risk for endometrioid and clear cell ovarian carcinoma. Endometrial stem cells are known to actively regenerate within endometrium and sheds into ectopic sites during retrograde menstruation which are suggested to be involved in the pathogenesis of endometriosis. A variety of molecular events have been correlated previously with the malignant transformation of endometriotic cyst such as alterations in genes

TP53, BCL-2, PTEN, ARID1A, CTNNB1. Unfortunately, early molecular events that contribute to tumor progression in EAC remain undefined.
Study design, size, duration: Endometrial (n = 18; P-EnSC) and endometriotic cyst (n = 11; EndoSC) biopsies were collected from patients undergoing surgery for endometriotic cystectomy. These patients were not under hormonal treatment or any intrauterine device for atleast three months prior to surgery. Also, endometrium from healthy fertile volunteers (n = 17; H-EnSC) was collected as control. Biopsies were enzymatically digested and sorted for MSC markers CD90, CD73 and CD105 by flowcytometry (FACS). MSC were characterized and used for analysis of putative CSC. subpopulations.
Participants/materials, setting, methods: *In vitro* monolayer cells from P-EnSC and P-EndoSC were compared against H-EnSC for cell cycle distribution. Also, they were checked for putative CSC population after growing them as 3D-multicellular spheroids for two generations and compared with H-EnSC for expression of CSC markers CD44, CD133, ALDH1A1, CD117, ABCG2; pluripotent markers OCT3/4, SOX2, NANOG using real-time-PCR and FACS. Colocalization of CSC markers were analysed using confocal imaging. Paired and unpaired t test were used for statistical analysis.
Main results and the role of chance: We observed an unique G1/S cycling cell-cycle distribution as previously reported with CSC, exclusively by increased early-S and late-S phase ($P < 0.05$) among both P-EnSCs and P-EndoSC on comparing H-EnSC with 5-Bromodeoxyuridine in DNA of newly dividing cells. Coincidentally, P-EnSC also showed higher expression of proliferative Ki67 marker and low expression of antiapoptotic gene BCL2 ($P < 0.05$) comparing H-EnSC indicating re-activation of a quiescent subpopulation. FACS sorting of P-EnSC and P-EndoSC with known endometrial and ovarian CSC markers CD44, CD133, ALDH1A1, CD117 and ABCG2 on monolayer cultured MSCs revealed existence of such rare CSC populations (~0.01–0.1%) in patient samples but they failed to survive in our long term culture. Hence, we enriched P-EnSC, P-EndoSC and H-EnSC into 3D-tumor spheroids which exhibited significant increase in expression of pluripotent markers OCT3/4, SOX2, NANOG, and CSC marker CD133/PROM1 between P-EnSC and H-EnSC spheroids ($P < 0.01$). Moreover, SOX2 were exclusively upregulated with P-EndoSC comparing P-EnSC, indicating possibility of dedifferentiation of MSC into CSC populations within endometrioma ($P < 0.05$, $P < 0.01$, $P < 0.01$, respectively). Further characterization by FACS between groups showed clonal expansion of $CD44^+CD133^+$ populations of P-EndoSC spheroids ($P < 0.01$). Validation with confocal imaging revealed increased expression of CSC markers $CD44^+CD133^+$ among P-EndoSC spheroids with higher colocalization index (0.91).
Limitations, reasons for caution: We included only endometriosis patients without any hormonal treatment. Although it improves the quality of results, it limits the overall sample size. Also, we tried to adopt a physiologically relevant *in vitro* model; however, an *in vivo* approach would have led to better understanding of transformation events leading to EAC.
Wider implications of the findings: Our study demonstrates for the first time the link between ovarian cancer and endometriosis at cellular level by identifying CSC phenotypes within both endometrium and endometriotic cyst. This would help in screening patients who exhibit CSC phenotypes and offer prophylactic measures to reduce their cancer risk.
Trial registration number: Not Applicable

O-026 Hypoxia and hypoxia inducible factor-1 in the human and murine endometrium: a role in the pathogenesis of prolonged, heavy menstrual bleeding

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Study question: Are hypoxic conditions and/or the activation of hypoxia inducible factor (HIF)-1α required for efficient endometrial repair at menstruation?
Summary answer: Hypoxia and hypoxia inducible factor-1α are required for efficient endometrial repair at menstruation and an aberrant hypoxic response results in prolonged, heavy menstrual bleeding.
What is known already: Heavy menstrual bleeding (HMB) is a common, debilitating condition that often requires surgical intervention. There is an unmet need for new, efficient medical therapies that lack hormonal side effects.
Following progesterone (P)-withdrawal in the late secretory phase, vasoconstriction of spiral arterioles is thought to lead to local hypoxia in the luminal endometrium. However, the presence and role of endometrial hypoxia has been the subject of intense debate. Hypoxia was shown to have no effect on endometrial breakdown but its role in subsequent repair (reepithelialisation and stromal expansion) remains unknown. Inefficient endometrial repair at menses may contribute to prolonged, HMB.
Study design, size, duration: Human case–control studies were performed with ethical approval and written consent to determine the presence of HIF-1α across the menstrual cycle in women with (>80 ml) and without (<80 ml) HMB (minimum n = 4 per group/cycle stage). A cohort design was utilised in murine studies to determine if lack of hypoxia or deficient HIF-1α was a risk factor for HMB (minimum of n = 6 per group).
Participants/materials, setting, methods: Endometrial biopsies were collected from healthy women. Those with fibroids, endometriosis, irregular cycles or taking hormones were excluded. Menstrual blood loss was objectively measured (modified alkaline-haematin method). HIF-1α was detected by Western blot and downstream targets by PCR.

Mice do not naturally menstruate. Ovariectomy, administration of estradiol/progesterone and induction of decidualisation enabled modeling of endometrial shedding/repair allowing manipulation of hypoxia and HIF in this *in vivo* model. Endometrial repair was quantified by histological grading.
Main results and the role of chance: HIF-1α was present in nuclear protein extracts from human endometrium, but limited to tissue exposed to P-withdrawal (perimenstrual). Women with HMB had significantly decreased endometrial HIF-1α at menstruation when compared to women with normal loss ($P < 0.05$). Downstream targets of HIF-1 (VEGF/CXCR4) were also decreased in women with HMB at menses ($P > 0.001$). Of note, women with HMB bled for 2 days longer on average than those with normal bleeding ($P < 0.01$).

We detected a transient hypoxic episode in murine endometrium during menstruation using pimonidazole (marker of O_2 <10 mmHg), which localised to the denuded surface. We prevented endometrial hypoxia during menses using a hyperoxic chamber (75% O_2), confirmed by absent pimonidazole staining. This unique model demonstrated menstrual hypoxia is necessary for (1) sufficient endometrial HIF-1α induction, with decreased HIF-1α protein in mice exposed to hyperoxia and (2) efficient endometrial repair, as evidenced by a decreased endometrial repair histoscore at 24 h in mice incubated in hyperoxia vs. normoxia ($P < 0.05$).

We genetically (HIF-1α heterozygote vs. wild type mice) or pharmacologically (i.p., injection of echinomycin vs. vehicle) reduced endometrial HIF-1α during menstruation in the mouse model of simulated menstruation. This also resulted in significantly delayed endometrial repair, due to aberrant endothelial cell function and delayed epithelial cell proliferation.
Limitations, reasons for caution: We acknowledge the number of human samples studied is small due to our strict classification and stringent exclusion criteria. The mouse model of simulated menses is not a natural model of menses but does mimic human endometrial changes and maintains endometrial architecture and cell–cell interactions critical for efficient menstruation/repair.
Wider implications of the findings: Herein, we demonstrate that hypoxia/markers of hypoxia are present in the human and murine menstrual endometrium. Prevention of this hypoxic episode, or of the subsequent stabilisation of HIF-1α, significantly delays endometrial repair. Correction of this defect could result in a novel, efficient medical treatment for women with HMB.
Trial registration number: N/A

SELECTED ORAL COMMUNICATIONS

SESSION 06: WHAT DOES GENOTYPE MEAN TO THE EMBRYO?

Monday 04 July 2016 **Hall 3 DE** **10:00–11:30**

O-027 Genetic diseases and aneuploidies can be detected with a single blastocyst biopsy: a new clinical successful approach.

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Study question: Is a single blastocyst biopsy efficient in detecting both genetic diseases and aneuploidies, limiting negative effect on embryo implantation potential and guaranteeing good clinical outcomes?
Summary answer: Genetic diseases and aneuploidies can be detected with a single biopsy without negative influence on blastocyst implantation and developmental potentials and allowing good clinical outcomes.
What is known already: Initially, preimplantation Genetic Diagnosis (PGD) was used in fertile patients who have the risk of transmitting genetically inheritable diseases to their offspring. Later, after the introduction of different techniques allowing the analysis of all the 24 chromosomes, the same procedure was applied to investigate the genetic status of the embryos (preimplantation Genetic Screening, PGS) obtained from infertile patients. The efficiency of performing routinely PGS in all the categories of patients with the aim to select the embryo(s) with the highest implantation potential, it is still an issue under intense debate in the literature.
Study design, size, duration: This consecutive case series study was performed from October 2011 to December 2015. Clinical and biological outcomes from 963 blastocysts obtained in 264 PGD cycles for monogenic diseases or translocations were analyzed. When the blastocyst resulted transferable after the PGD analysis, also its ploidy status by mean of PGS was detected using the same biopsy sample. Mean female age was 35.26 ± 4.16 years old. All biopsies were performed at blastocyst stage with array comparative genomic hybridization.
Participants/materials, setting, methods: All mature oocytes retrieved were injected and cultured individually until the blastocyst stage at 37°C, 6% CO_2, 5% O_2. When the blastocyst was formed, it was biopsied and vitrified, waiting for the genetic results. The frozen–thawed embryo-transfer was performed in a subsequent cycle. Sometimes, when the blastocyst was obtained within the morning of day-5 of culture, it has been maintained in culture in order to perform a fresh embryo-transfer on day-6, after receiving the genetic report.
Main results and the role of chance: A total of 2389 mature oocytes were injected with a fertilization rate of 75.1% (N = 1795); 1780 embryos were obtained with a blastocyst formation rate of 54.1% (N = 963); 952 of them were biopsied from day-4 to day-7 of culture. After the genetic analysis, 269 blastocysts resulted transferable, both for monogenic disease or translocation and for their ploidy status, 31 mosaic, 52 no result and 600 not transferable, for the genetic disease and/or for their ploidy status. A total of 174 embryo-transfer were performed, 53 fresh and 121 cryopreserved, where 184 healthy or carrier euploid blastocysts were transferred. In cryopreserved embryo-transfer, 130 out of 135 (96.3%) warmed blastocysts survived. The clinical pregnancies, the heart beats, the miscarriages and the ectopic pregnancies were 82 (47.1%), 85 (implantation rate of 46.2%), 17 (9.8%) and 1 (0.6%), respectively. To date, 53 deliveries occurred whit 56 healthy babies born, 28 males and 28 females; 11 pregnancies are still ongoing. In addition, there are still 111 transferable blastocysts cryopreserved obtained from 49 cycles, that could lead to further improved clinical outcomes. Finally, it will be possible to perform a second biopsy on the no results blastocysts, with the hope to recover other transferable ones.
Limitations, reasons for caution: A higher cycle cancellation rate than expected could be found due to the double kind of genetic analysis performed. For this reason, particular care should be taken in drafting and explaining informed consent, and in particular the risks related to this approach, in order to avoid patients' drop out.
Wider implications of the findings: When the biopsy have to be performed in order to prevent the transmission of a hereditary disease, it should be mandatory to analyze also the genetic status of the blastocyst, avoiding useless embryo-transfers in this particular category of patients. Blastocyst seems to be the more convenient stage to perform biopsy.
Trial registration number: Not applicable

O-028 Clinical outcome derived after tranfer of embryos with chromosomal mosaicism

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Study question: Can optimized protocol for chromosomal mosaicism determination be applied for aneuploidy screening of human embryos to detect potentially transferrable mosaic embryos?
Summary answer: The adoption of optimized protocol improves the detection of embryos with chromosomal mosaicism and identification of embryos capable to implant and result in normal pregnancies.
What is known already: Aneuploidy screening of embryos at blastocyst stage can be jeopardized by the presence of chromosomal mosaicism, a phenomenon characterized as a mixture of diploid and aneuploid cell lines in the same embryo. Although many of such embryos do not implant, some may undergo through a natural mechanism of aneuploidies rescue during development resulting in euploid embryos. However, technical limitations have precluded accurate detection of mosaicism after preimplantation genetic screening of embryos preventing, in some case, transfer of only viable mosaic embryos (false positive) or, in the opposite scenario, transferring undetected mosaic embryos (false negative) instead of, if available, euploid embryos.
Study design, size, duration: This study was organized into two steps. The first involved the analysis of 195 experimental samples composed with different euploid and aneuploid single cell ratios (10–90%) to obtain reference curves for chromosomal mosaicism. In the second step, embryos obtained from 1525 PGS cycles, performed from May 2013 to April 2015 were analysed. The clinical outcome (implantation, miscarriage, and live birth rate) after euploid and mosaic embryos transfer was evaluated.
Participants/materials, setting, methods: All embryos were cultured to blastocyst stage; trophectoderm biopsy was performed on day-5 of development. Experimental and clinical samples were analysed with array-comparative genomic hybridization (array-CGH) and Next-Generation Sequencing (NGS) methodologies accomplished by BlueFuse Multi (BFM) analysis software. Percentage of chromosomal mosaicism was determined based on reference curves in both mosaic and full aneuploidy embryos. Designated mosaic embryos were offered to patients without euploid embryos available.
Main results and the role of chance: Based on reference curves 2191 euploid, and 3019 aneuploid embryos were detected. The remaining 452 were classified as mosaic embryos including 117 (13%) of samples predicted to be uniformly aneuploidy by BFM automatic call. Following transfer of 993 euploid embryos in 941 women (mean age 38.4 years range 20–48), 610 had positive hCG levels: 507 pregnancies continued, confirmed by at least one fetal sac and heart beat (54% clinical pregnancy rate/ET), 95 were biochemical and 46 miscarried. Following transfer of 39 mosaic embryos in 38 women (mean age 37 ± 4 years, range 29–44), 13 resulted in healthy babies (33.3%). Of these, 7(54%) were from blastocysts with 40% mosaicism, 4 (31%) from 50% and 2 from blastocysts with 30% mosaicism. Twelve of babies born (92.4%) were from blastocysts with a single or double monosomies, one (7.6%) was from a single trisomy.
Limitations, reasons for caution: Although clinical results have documented high pregnancy outcomes following transfer of mosaic embryos scored with a chromosomal mosaicism curve, a randomized controlled trial confirming its clinical effectiveness is advisable before recommending widespread application.
Wider implications of the findings: Our results indicate that adoption of a proper reference curve able to discriminate full euploid or aneuploidy from mosaic embryos may represent a strategy to avoid the discarding of viable embryos and offer IVF-patients with only aneuploid embryos, a chance of success
Trial registration number: No external funding was sought for this study

O-029 The clinical significance of segmental aneuploidy in human oocytes and preimplantation embryos

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Study question: What is the incidence, origin and significance of segmental aneuploidy in oocytes and embryos? Does the presence of partial chromosome errors affect embryo implantation ability?
Summary answer: Segmental aneuploidy is frequent in embryos. The nature of such abnormalities should be considered when selecting embryos for transfer, after preimplantation genetic screening (PGS).

What is known already: PGS has been proposed as a tool for embryo selection, distinguishing embryos affected by lethal aneuploidies from those that are euploid and potentially viable. The adverse impact of aneuploidy involving entire chromosomes, in terms of reduced likelihood of embryo implantation and increased miscarriage risk, is well-established. However, losses or gains of chromosomal fragments, resulting in segmental aneuploidies, are also common during preimplantation development. The clinical relevance of segmental aneuploidies is unclear at this time. Given the high frequency of segmental aneuploidy, investigations to determine the impact of this class of abnormality in the context of IVF outcome are urgently required.
Study design, size, duration: Cytogenetic data was obtained from oocytes and embryos of 690 IVF patients who requested PGS for various reasons, most commonly advanced female age or previously unsuccessful IVF treatments. A total of 4,068 samples including 452 human oocytes, 1,726 cleavage stage and 1,890 blastocyst stage embryos were investigated. The data was obtained over a period of 3 years, from 2012 to 2015.
Participants/materials, setting, methods: Both the first and second polar bodies were examined from the oocytes included in this study. Analysis at the cleavage stage involved testing of single blastomeres, while blastocysts were assessed by investigating trophectoderm biopsy specimens (~5 cells). The biopsied material was subjected to whole genome amplification followed by comprehensive chromosome analysis using well-validated microarray comparative genomic hybridisation (aCGH) or next generation sequencing (NGS) methods.
Main results and the role of chance: Segmental aneuploidy was identified in 10.39% of oocytes (47/452), 24.29% of cleavage stage embryos (428/1726) and 15.59% of blastocysts (207/1327). Thus, the incidence of segmental aneuploidy increased dramatically after fertilisation ($p < 0.0001$), but declined from the cleavage to the blastocyst stage ($p < 0.0001$). The high levels of segmental abnormality at the cleavage stage, relative to levels seen in oocytes, suggests that most segmental aneuploidies are derived from the sperm, or spontaneously arise during the first few mitotic divisions post-fertilisation. Approximately 75% of the chromosomes breaks associated with segmental aneuploidies occurred along the chromosome arms, while ~25% occurred at or near centromeres. Additionally, distinct chromosome breakage hotspots were found at specific locations on individual chromosomes, some corresponding to known chromosomal fragile sites. The NGS method utilised is able to detect mosaic chromosome abnormalities with high sensitivity (i.e., aneuploidy restricted to a subset of the biopsied cells). Analysis of 563 trophectoderm biopsies using NGS revealed that three-quarters of segmental abnormalities were present in mosaic form (122 vs. 43, respectively). In some cases embryos with mosaic abnormalities were transferred. Clinical follow-up conclusively demonstrated that embryos with mosaic segmental errors had a higher chance of producing ongoing pregnancies than those with mosaic for whole chromosomal aneuploidy ($p = 0.0021$).
Limitations, reasons for caution: The scoring of segmental aneuploidy using different techniques for PGS (such as aCGH, real-time PCR and NGS) across different laboratories is currently without consensus. This issue needs to be addressed. Some widely used PGS methods are unable to accurately detect segmental abnormalities, leading to contradictory reports in the literature.
Wider implications of the findings: Understanding the impact of segmental aneuploidies will lead to improved clinical management. Data on the origin of the defect (meiotic/mitotic), and the site of chromosomal breakage, can provide an indication of the likelihood of mosaicism. This information is relevant to embryo viability and the potential to produce a healthy child.
Trial registration number: Not applicable

O-030 Embryos showing mosaicism in trophoectoderm cells can achieve good pregnancy rates

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Study question: Should embryos carriers of mosaicism in trophoectoderm cells be discarded?
Summary answer: Transfer of mosaic embryos leads to a lower but relatively good clinical pregnancy rate.
What is known already: There is a high incidence of chromosomal abnormalities in human embryos that leads to failure of IVF cycles. The chromosomal mosaicism, which consists of a mixture of diploid and aneuploid cells, is a common phenomenon in IVF-derived embryos. Classification of mosaic embryos as aneuploid embryos is a controversial topic, since the association between mosaicism in trophoectoderm cells and inner mass cells is unknown. Moreover, it seems that there is a mechanism by which mosaicism could be corrected. To clarify this issue the outcomes of transfer cycles with mosaic and euploid blastocysts were compared.
Study design, size, duration: We retrospectively reanalyzed array-CGH results from trophoectoderm biopsies of day 5 and 6 blastocysts (from October 2014 to December 2015). A total of 816 embryos were included. We considered a mosaic embryo when the percentage of mosaicism, calculated by the log2 ratio, was higher than 25%. The mosaic embryos ($n = 107$) were classified in two groups according to the number of chromosomes with mosaicism: 1 chromosome ($n = 48$) and two or more chromosomes ($n = 59$). **Participants/materials, setting, methods:** Chromosomal comprehensive screening was performed to couples who attended the Instituto Bernabeu for advanced maternal age, abnormal sperm FISH and/or a history of recurrent miscarriage or implantation failure. Array-CGH analysis was performed using Agilent SurePrint G3 8 × 60 K CGH microarrays with previous whole genome amplification of genomic DNA. The main outcome measures were implantation rate, pregnancy rate and biochemical and clinical miscarriage rate. The differences between groups were evaluated using the Fisher's exact statistical test (SPSSv20.0).
Main results and the role of chance: The array-CGH results from trophoectoderm biopsies of day 5 and 6 blastocysts ($n = 816$) were reanalysed. We detected chromosomal mosaicism in 107 blastocysts (13.1%). Moreover, 49.4% of the analysed embryos were euploid, 30.9% aneuploid and the remaining 6.6% without diagnosis. In the mosaic group, 57.9% were euploid embryos with mosaicism and 42.1% were also aneuploid. In the euploid-mosaic embryos, the frequency of embryos with mosaicism in only 1 chromosome was 51.6% vs. 48.4% for those with mosaicism in 2 or more chromosomes.

The outcomes of the cycles were compared between cycles where only mosaic embryos were transferred and cycles where euploid embryos were transferred. Although the outcomes of transfer cycles seem to be lower among the mosaic group, the differences with regard to pregnancy rate, implantation rate, miscarriage rate and ongoing pregnancy rate were considered not quite statistically significant. However, significant differences were observed in the clinical pregnancy rate (20.6% in mosaic group vs. 38.9% in euploid group; $p = 0.042$). In the mosaic group, according to the number of chromosomes with mosaicism, the embryos with mosaicism in 2 or more chromosomes showed a lower pregnancy rate compared with the embryos with mosaicism in 1 chromosome (23.5% vs. 64.7%; $p = 0.016$).
Limitations, reasons for caution: It is unknown if the mosaicism detected in a biopsied embryo is confined only to trophoectoderm, and moreover if the embryo is able to correct it. More data are needed to conclude which mechanisms are involved in the development of these mosaic embryos.
Wider implications of the findings: Our data show that the transfer of mosaic embryos affects the outcomes of the IVF cycles. Even so, the mosaic embryos have a relatively good IVF success rate and therefore they should not be discarded in couples that don't have euploid embryos for transfer.
Trial registration number: No clinical trial

O-031 Incidence, origin and type of aneuploidy seen in human preimplantation blastocysts: concurrent analysis using single nucleotide polymorphism (SNP) arrays and next generation sequencing (NGS)

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Study question: What is the incidence and origin of aneuploidy in human preimplantation blastocysts and what are the types of aneuploidies observed?
Summary answer: Aneuploidy of maternal meiotic origin was most common and displayed an equal distribution of monosomies and trisomies. Paternal-derived aneuploidy was mostly associated with chromosome loss.
What is known already: The incidence of aneuploidy in human gametes and embryos has been studied in the past and has been shown to be strongly correlated with increasing maternal age. It is known that aneuploidy in human embryos can be of meiotic (gamete) origin or be mitotic (arising after fertilization). In the past, phenomena and processes contributing to meiotic and mitotic

chromosome errors have tended to be studied separately. A comprehensive investigation looking into all the different types of aneuploidy simultaneously, and within the same cohort of samples, could provide valuable data, offering a thorough insight into this biologically and clinically important phenomenon.
Study design, size, duration: Single nucleotide polymorphism (SNP) arrays and next generation sequencing (NGS) were used in parallel to screen over 8,700 chromosomes from 190 *in vitro* fertilized embryos (blastocysts). These embryos were generated by 31 couples (average maternal age 33.87 ± 0.80 years) undergoing preimplantation genetic diagnosis (PGD) for single gene disorders in conjunction to comprehensive chromosome screening. The SNP arrays were used for PGD *via* a linkage-based (Karyomapping, Illumina, USA) approach, while NGS was used for aneuploidy detection.
Participants/materials, setting, methods: Embryos were biopsied at the blastocyst stage and the trophectoderm samples obtained were subjected to multiple displacement amplification. Karyomapping was then used to generate data from 300,000 SNPs per embryo. Next generation sequencing was carried out using the VeriSeq PGS assay (Illumina). A MiSeq desktop sequencer was utilized for NGS. BlueFuse Multi analysis software was used for interpretation of results obtained from Karyomapping and VeriSeq PGS assays.
Main results and the role of chance: Results were generated from 179 embryos of which 54.8% were identified as containing at least one chromosomal abnormality; 45.4% of the embryos exhibited only meiotic abnormalities, 39.2% had only mitotic abnormalities and the rest had both types of aneuploidy. Of a total of 164 aneuploidy events, 52.4% were determined to be meiotic in origin and 47.6% mitotic in origin. Maternal meiotic events (81.4%) eclipsed paternal meiotic events (18.6%). While maternal meiosis consisted of an equal number of events leading to gain and loss of chromosomes, paternal meiotic errors were more likely to involve loss of chromosomes (93.75% vs. 6.25%), with almost half of these being partial losses.

We also looked into the incidence of meiotic errors according to maternal age (calculated as No. of embryos with meiotic errors vs. overall No. of embryos per age group). It was determined that meiotic errors increase with increasing female age with highest predominance being at ages ≥40 years. Specifically, the incidence of meiotic errors was determined to be 14.2% for <34 age group, 26.4% for 35–39 age group and 73.7% for ≥40 years; incidence was significantly higher for ages 40 years ($P < 0.001$). However, mitotic errors did not increase with age.
Limitations, reasons for caution: In order to have robust data concerning the incidence of aneuploidy affecting individual chromosomes, an even larger dataset would be required. The current research can only be used to assess general trends.
Wider implications of the findings: Maternal meiotic errors dominated and increased with age, explaining the higher rates of implantation failure and miscarriage experienced by older women. Mitotic errors, potentially producing mosaicism, were also common. Interestingly, aneuploidy of male meiotic origin was characterized by a preponderance of monosomies, suggesting a different mechanism to their female counterparts.
Trial registration number: Non-Applicable

O-032 Mutations in TUBB8 cause a multiplicity of phenotypes in human oocytes and early embryos

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Study question: What phenotype of human oocytes and early embryos can caused by TUBB8 mutations
Summary answer: *TUBB8*mutations can cause oocyte meiotic arrest or embryos developmental arrested by either dominant negative effects or tubulin deficiency
What is known already: TUBB8 is unusual in that it exists only in primate species, where its biological function was unknown. Recently, we identified seven missense mutations in TUBB8 in patients with oocyte maturation arrest. We found that TUBB8 is uniquely expressed in oocytes and the early embryo, where it is the preponderant β-tubulin isotype. Based on an assessment of their effects upon expression in HeLa cells, yeast cells, mouse and human oocytes, we concluded that these mutations exert their effects *via* dominant negative effects on microtubule behavior. These findings uncovered an essential role for TUBB8 in human oocyte maturation and female fertility.
Study design, size, duration: Patients with oocyte maturation arrest were referred from the reproductive medicine center at Ninth Hospital affiliated with Shanghai Jiao Tong University and Shanghai Ji Ai Genetics from January 2014 to December 2015.
Participants/materials, setting, methods: Patient genomic DNA samples and those from their family members and controls were extracted. All exons and splicing sites of *TUBB8* were amplified and sequenced. Oocytes obtained from donors undergoing routine clinical ICSI were viewed by phase contrast and polarization microscopy with an OLYMPUS IX71 inverted microscope system. Folding kinetics of wild type and mutant were evaluated and transfected into HeLa cells to evaluate microtubule phenotypes
Main results and the role of chance: We report eight new inherited or de novo *TUBB8*mutations and a recurrent de novo *TUBB8* mutation that cause female infertility in nine independent families. Seven of these (S176L, I210V, T238M, V255M, R262W, T285P, and N348S) are missense, and are structurally predicted to interfere with various aspects of microtubule behavior. With the exception of R262W, these mutations cause varying degrees of microtubule disruption upon expression in cultured cells as a result of dominant negative effects. In spite of lacking a detectable spindle or containing an impaired spindle, oocytes harboring three of these mutations (I210V, T238M and N348S) can extrude the first polar body. Moreover, they can be fertilized, although the ensuing embryos become developmentally arrested. These data underscore the independent nature of human oocyte meiosis and differentiation. We also describe two infertile patients carrying homozygous *TUBB8*mutations (p.T143Dfs*12 and p.(E27_A33del)) that render the protein folding and assembly incompetent. Surprisingly, oocytes from these patients have identifiable spindles, although in at least one case these had an abnormal morphology. These observations imply that such spindles are assembled from pre-existing non-TUBB8 tubulin heterodimers expressed at an earlier developmental stage, and that tubulin deficit can contribute to meiotic arrest.
Limitations, reasons for caution: TUBB8 mutations need to be evaluated in larger sample of patients with human oocyte meiotic arrest. Corresponding oocytes and embryos phenotypes incurred by TUBB8 mutations should be deeply evaluated
Wider implications of the findings: Currently, in IVF/ICSI clinics, only morphologically MII oocytes identified by extrusion of the first polar body are selected for fertilization. Our observations provide an additional criterion that can be applied for evaluating the quality of oocytes selected for fertilization in the future.
Trial registration number: Not applicable

SELECTED ORAL COMMUNICATIONS

SESSION 07: OPTIMIZING OVARIAN STIMULATION

Monday 04 July 2016 **Room 101** **10:00–11:30**

O-033 Results of the ESPART randomized controlled trial investigating recombinant luteinizing hormone supplementation for controlled ovarian stimulation in poor ovarian responders aligned with the Bologna criteria

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Study question: Does fixed-ratio combination recombinant-human follicle-stimulating hormone (r-hFSH) plus recombinant-human luteinizing hormone (r-hLH) increase the number of oocytes vs. r-hFSH in Bologna criteria-aligned poor ovarian responders?
Summary answer: No significant difference in the number of oocytes retrieved between poor ovarian responders receiving fixed-ratio combination r-hFSH plus r-hLH and those receiving r-hFSH was seen.

What is known already: At present no "gold standard" protocol for controlled ovarian stimulation (COS) in women with poor ovarian response (POR) exists; nevertheless, evidence from previous meta-analyses have suggested that the addition of r-hLH to r-hFSH may be beneficial for COS in this population. However, clinical trials included in the meta-analyses used a variety of definitions for POR, reducing their comparability. Subsequently, the ESHRE Bologna criteria have been set, in an attempt to identify a standardized population for inclusion in clinical trials investigating POR.
Study design, size, duration: The Efficacy and Safety of Pergoveris in Assisted Reproductive Technology (ESPART) study is a phase III, randomized, single-blind, parallel-group, active-comparator trial, comparing the number of oocytes retrieved following COS in 939 women randomized (1:1) to receive either fixed-ratio r-hFSH plus r-hLH (2:1 ratio) or r-hFSH monotherapy. It is the largest RCT to date in patients with POR and includes follow-up to the assessment of live birth rate, over a single assisted reproductive technologies (ART) cycle.
Participants/materials, setting, methods: Patients meeting ≥2 of the following (aligned to ESHRE Bologna criteria) were included: age >40–<41 years; previous cycle with ≤3 oocytes retrieved with conventional stimulation; anti-Müllerian hormone (AMH) level 0.12–1.3 ng/mL. Following downregulation, 462 women were randomized to COS with r-hFSH/r-hLH and 477 to COS with r-hFSH. The primary endpoint was number of oocytes retrieved. Secondary and other outcomes included biochemical, clinical and ongoing pregnancy rates, embryo implantation rate and live birth rate.
Main results and the role of chance: The mean age of patients was 38.3 years, mean AMH level was 0.59 ng/mL, and mean antral follicle count was 4.8. 83% of patients had at least one previous ART cycle with ≤3 oocytes retrieved. In the overall Modified Intention-to-Treat population (and supported by the Per Protocol population), there was no significant difference in the mean [standard deviation] number of oocytes retrieved in the r-hFSH plus r-hLH group (3.3 [2.71]) compared with the r-hFSH group (3.6 [2.82]; $P = 0.054$). The biochemical pregnancy rate was significantly lower in the r-hFSH plus r-hLH group compared with the r-hFSH group (17.3% vs. 23.9%, respectively), however, no significant difference was seen between the groups for clinical pregnancy rate (14.1% vs. 16.8%), ongoing pregnancy rate (11.0% vs. 12.4%) and live birth rate (10.6% vs. 11.7%). The incidence of adverse events was comparable between the treatment groups, occurring in 25.8% and 33.3% of women treated with r-hFSH/r-hLH and r-hFSH, respectively, and no new safety concerns were identified. When subgroups of patients were investigated according to the individual Bologna criterion defined in the study (age, AMH level and ART history) separately, no subpopulation was identified that benefitted from the addition of r-hLH to r-hFSH.
Limitations, reasons for caution: Although a definition of POR aligned with the ESHRE Bologna criteria was used, some degree of clinical heterogeneity still existed within the population which may have impacted the outcome.
Wider implications of the findings: This study demonstrated that, in the worst prognosis patient, with POR aligned with the Bologna criteria, no benefit from fixed-ratio r-hLH supplementation during COS was seen.
Trial registration number: ClinicalTrials.gov identifier: NCT02047227; EudraCT Number: 2013-003817-16.

O-034 Are AMH level and AFC useful predictors of outcomes in poor ovarian responders? A post hoc analysis of the ESPART randomized controlled trial

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Study question: Are anti-Müllerian hormone (AMH) levels and antral follicle count (AFC) associated with outcomes in poor ovarian responders (PORs), aligned with the Bologna criteria?
Summary answer: In PORs, AMH levels and AFC were associated with the number of oocytes obtained, but not with ongoing pregnancy or live birth rates.
What is known already: In the general infertility population undergoing assisted reproductive technologies (ART) both AMH level and AFC are associated with the number of oocytes retrieved. This association has not previously been investigated in Bologna PORs. The ESPART study aimed to demonstrate superiority of a fixed-dose combination r-hFSH + r-hLH compared with r-hFSH in women with poor ovarian response aligned with the Bologna criteria (≥2 of the following: >40–<41 years; previous cycle with ≤3 oocytes retrieved with conventional stimulation; AMH level 0.12–1.3 ng/mL). No difference was observed between groups with regards to number of oocytes retrieved, ongoing pregnancy rates and live birth rates.
Study design, size, duration: Regression models investigating the number of oocytes retrieved, ongoing pregnancy and live birth rates were developed using ESPART study data. For the number of oocytes retrieved, a Poisson regression model was used with AMH level, AFC and age as explanatory variables. Logistic regression models for ongoing pregnancy rate and live birth rate included these terms and also the number of oocytes retrieved as explanatory variables. Association between terms was investigated using the Pearson correlation coefficient.
Participants/materials, setting, methods: The intention-to-treat population from ESPART was used (939 patients; r-hFSH + r-hLH, $n = 462$; r-hFSH, $n = 477$). The mean age was 38.3 years, the mean AMH level was

0.59 ng/mL and the mean AFC was 4.8. The mean number of oocytes retrieved was 3.3 and 3.6 in patients receiving r-hFSH + r-hLH and r-hFSH, respectively. There were 51/462 (11.0%) and 59/477 (12.4%) ongoing pregnancies with r-hFSH + r-hLH and r-hFSH, respectively, and 49/462 (10.6%) and 56/477 (11.7%) live births, respectively.
Main results and the role of chance: In this population of PORs ($n = 939$), the AMH level and AFC were strongly associated with the number of oocytes retrieved ($P < 0.0001$ for both), whereas age was not ($P = 0.082$). The AMH level was associated with AFC (Pearson correlation coefficient = 0.346; $P < 0.0001$). Age and the number of oocytes retrieved were strongly associated with ongoing pregnancy rate ($P = 0.003$ and <0.0001, respectively) and live birth rate ($P = 0.006$ and <0.0001, respectively), whereas AMH level and AFC were not associated with ongoing pregnancy rates ($P = 0.102$ and 0.096, respectively) or live birth rates ($P = 0.163$ and 0.096, respectively). Overall there was a trend for higher ongoing pregnancy and live birth rates in patients with AMH levels >0.5 and ≤1.1 ng/mL. Ongoing pregnancy rates were 9.8% (46/472), 14.2% (52/366) and 10.5% (10/95) in patients with AMH levels ≤0.5, >0.5–≤1.1 and >1.1 ng/mL, respectively; live birth rates were 9.3% (44/472), 13.7% (50/366) and 9.5% (9/95), respectively. Likewise, when only patients with AMH levels of ≤1.1 ng/mL were included ($n = 838$), comparable outcomes were obtained from the regression models, with AMH levels and AFC being associated with number of oocytes retrieved ($P < 0.0001$ for both) but not with ongoing pregnancy ($P = 0.137$ and 0.339, respectively) or live birth rates ($P = 0.212$ and 0.292, respectively).
Limitations, reasons for caution: Although a regression model can indicate associations between baseline characteristics and outcomes, this does not confirm a causal relationship. Furthermore, the ESPART study inclusion criteria were aligned with the Bologna criteria, but not identical to them. Therefore, these results may not be applicable to the strict Bologna criteria POR population.
Wider implications of the findings: This analysis suggests that although AMH level and AFC may provide guidance on controlled ovarian stimulation outcomes, in this population they do not predict the live birth rate. Age, however, was related to live birth rate and, therefore, should be considered when informing patients about outcomes.
Trial registration number: ClinicalTrials.gov identifier: NCT02047227; EudraCT Number: 2013-003817-16.

O-035 Optimization of outcome through individualized dosing in predicted poor responders undergoing IVF/ICSI; the OPTIMIST randomized controlled trial, NTR2657

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Study question: Does an individualized dose of gonadotropin based on the antral follicle count (AFC) improve live birth rates in predicted poor responders undergoing IVF/ICSI?
Summary answer: Individualized dosing of gonadotropin based on the AFC does not improve the 18 months cumulative live birth rate in predicted poor responders.
What is known already: It is common practice to adjust the gonadotropin dose when a poor response is predicted based on ovarian reserve tests or after a poor response in a previous cycle. However, evidence such dose adjustments is limited.
Study design, size, duration: A multi-center cohort study in women starting their first IVF/ICSI cycle. Women with a predicted poor response (AFC < 11 follicles sized 2–10 mm) were randomized between an adjusted (225 (AFC 8–10) or 450 IU FSH (AFC < 8)) and standard dose of 150 IU FSH. Primary outcome was ongoing pregnancy leading to live birth <18 months after randomization. We needed to include 300 women to demonstrate a 15% gain in live birth rate (two-sided alpha-error 0.05, power 80%).
Participants/materials, setting, methods: Of 1503 women included in the cohort, 501 (33%) were predicted poor responders of which 238 were randomized to an adjusted (N = 107 received 450 IU FSH; N = 131 received 225 IU FSH) and 263 women to a standard dose. Following strict, predetermined criteria, limited dose adjustment in subsequent cycles was allowed in the standard treatment group only.

Pregnancies achieved <18 months after randomization and that are still ongoing at present are considered as live births (N = 39).
Main results and the role of chance: Women were included between May 2011 and May 2014. Baseline characteristics were comparable amongst groups. The rate of cumulative ongoing pregnancies leading to live birth rate from any transfer of fresh or frozen/thawed embryos within 18 months did not differ between adjusted and standard treatment (41% vs. 41%, corresponding to a RR of 1.0 [CI 95% 0.8–1.2]). Average time to positive pregnancy test leading to live birth was comparable in both groups (5.1 months for the adjusted group vs. 4.5 months in standard treatment, p = 0.334)

In first cycles (fresh and frozen/thawed embryos), no difference between groups was seen in live birth rate (18% in adjusted treatment vs. 18% in standard treatment, RR 1.0[95% CI 0.7–1.5]). A higher number of oocytes was retrieved in the adjusted dose group (7.6 vs. 6.5, mean difference 1.1[95% CI of the difference 0.2–2.0]). Also, in the first cycle, overall cycle cancellation rate was lower in the adjusted treatment group (8% vs. 23% in the standard treatment group, RR 0.3 [95% CI 0.2–0.6]). First cycles were mostly cancelled due to a poor response; 7% in the adjusted treatment group vs. 21% in the standard treatment group, RR 0.32 [95% CI 0.19–0.54].
Limitations, reasons for caution: Although we provided ultrasound training and guidelines, no quality control system was operational for AFC-measurements. Despite reported sufficient inter-observer reproducibility, categorization may have been imprecise. Stratified randomization, however, will have ensured comparability.

Secondary outcomes were based on first cycle data. Complete cumulative data will be available at the ESHRE meeting.
Wider implications of the findings: AFC-based individualized gonadotropin dosing in predicted poor responders does not improve live birth rates and should therefore not be clinical practice. In the first cycle, adjustment led to more oocytes and fewer cancellations, without influencing live birth rates or time to pregnancy, suggesting extra oocytes do not produce quality advantage.
Trial registration number: Registered at the "Dutch Trial Registry" (www.trialregister.nl). Registration number: NTR2657.

O-036 Optimization of outcome through individualized dosing in predicted hyper responders undergoing IVF/ICSI; The OPTIMIST randomized controlled trial, NTR2657

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Study question: Does an individualized dose of gonadotropins based on the antral follicle count (AFC) improve live birth rates in predicted hyper responders undergoing IVF/ICSI?
Summary answer: Gonadotropin dose adjustment in predicted hyper responders does not influence live birth rates, while it increased first cycle cancellations for a reduction in mild/moderate OHSS.
What is known already: Exaggerated ovarian response to gonadotropin stimulation for IVF may result in increased rates of cycle cancellation, the occurrence of complications such as the ovarian hyperstimulation syndrome (OHSS), and even slightly jeopardize live birth rates. In daily practice, lower gonadotropin doses are often administered to predicted hyper responders for safety reasons. However, no knowledge is available on whether such individualized gonadotropin dosing does indeed improve safety and increase efficacy and efficiency compared to a standard dose.
Study design, size, duration: A nationwide, multi-center cohort study in women scheduled for a first IVF/ICSI treatment. Women with a predicted hyperresponse based on their AFC (>15 follicles) were invited to participate in an RCT comparing an adjusted dose (100 IU FSH) to standard treatment (150 IU FSH). Since this RCT was embedded in a cohort study assessing over 1500 women, we expected to randomize 300 couples. Primary outcome was ongoing pregnancy leading to live birth within 18 months after randomization.
Participants/materials, setting, methods: Women were included between May 2011 and May 2014. From the 1503 women included in the cohort study, in 531 (35.3%) a hyperresponse was predicted and they were randomized to receive the adjusted fixed dose (100 IU, N = 258) or standard fixed dose; (150 IU, N = 273). Following strict, predetermined criteria, small dose adjustments were allowed in subsequent cycles.

Pregnancies achieved <18 months after randomization and that are still ongoing at present, are considered as live births (N = 39).
Main results and the role of chance: Baseline characteristics were comparable. The primary outcome, ongoing pregnancy rate from any transfer of fresh or frozen/thawed embryos leading to live birth <18 months after randomization, was 68% in the adjusted vs. 68% in the standard group (RR 1.0[95% CI 0.89–1.12]). Average time to positive pregnancy test leading to live birth was comparable in both groups (4.6 vs. 4.6 months, p = 0.749). In the first cycle (fresh and frozen/thawed embryos), live birth rates were 33% vs. 37% (RR 1.1(95% CI 0.9–1.4]).

In firsts cycles, the number of retrieved oocytes was significantly lower in the adjusted group (8.9 and 13.3, mean difference −4.4[95% CI−5.4–3.3]). Overall cycle cancellation rate in the first cycle was higher in the adjusted dose group (25% vs. 13%, RR 1.94[95% CI 1.3–2.8]). Cancellation rates for poor response were 22% vs. 3% (RR 6.8[95% CI 3.5–13.5]) and cancellation rates for hyperresponse were 2% vs. 8% (RR 0.2[95% CI 0.1–0.6]). After the first cycle, mild OHSS occurred in 3% of women in the adjusted and in 12% of women in the standard group (RR 0.27[95% CI 0.13–0.57]), while moderate OHSS did not occur in the adjusted group compared to 3% in the standard

group (RR 0.01[95% CI 1.01–1.05]). Incidence of severe OHSS was comparable (1.2% vs. 1.1%).

Limitations, reasons for caution: Although we provided ultrasound training and guidelines, no quality control system was operational for AFC-measurements. Despite reported sufficient inter-observer reproducibility, categorization may have been imprecise. Stratified randomization, however, will have ensured comparability.

Secondary outcomes were based on first cycle data. Complete cumulative data will be available at the ESHRE meeting.

Wider implications of the findings: In women with a predicted hyperresponse scheduled for IVF/ICSI, an adjusted dose does not affect live birth rates or time to pregnancy, despite a clearly reduced ovarian response. While dose adjustment limited the occurrence of mild/moderate OHSS, it also increased the cancellation rate and therefore cost-effectiveness should be further assessed.

Trial registration number: Registered at the "Dutch Trial Registry" (www.trialregister.nl). Registration number: NTR2657.

O-037 Cost-effectiveness of ovarian reserve testing in an IVF program; the OPTIMIST study

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Study question: Is ovarian reserve testing with the antral follicle count (AFC) and subsequent dose adjustment of gonadotropins cost-effective in women undergoing IVF/ICSI?

Summary answer: Ovarian reserve testing with the AFC and subsequent dose adjustment of gonadotropins is not more cost-effective than a standard dosing strategy in women undergoing IVF.

What is known already: While it has become common practice to assess ovarian reserve in women scheduled for IVF/ICSI and subsequently adjust the gonadotropin dose in case of a predicted poor or hyperresponse, it is unknown whether such a policy is cost-effective.

Study design, size, duration: We performed a multi-center cohort study with two embedded clinical trials in women starting their first IVF/ICSI cycle. We then developed a model comparing costs and effects of two strategies: (I) AFC measurement with gonadotropin dose adjustment (ORT strategy) or (II) standard dosing (SD) strategy. We calculated the number of live births, the number of cycles, the amount of FSH used and the number ovarian reserve tests performed for both strategies.

Participants/materials, setting, methods: Women with a predicted poor response (AFC < 11 follicles sized 2–10 mm) were randomized between an adjusted (225 (AFC 8–10) or 450 IU FSH (AFC < 8)) and standard dose of 150 IU FSH, while women with a predicted hyper response (AFC > 15) were randomized between an adjusted (100 IU FSH) and standard dose of 150 IU FSH. Women with a predicted normal response (AFC 11–15) received a standard dose of 150 IU FSH.

Main results and the role of chance: Between May 2011 and May 2014, we included 1503 women in the cohort, of whom 471 (31%) had a predicted normal response, 501 (34%) a predicted poor response and 531 (35%) a predicted hyper response. Both the ORT and SD strategy resulted in comparable ongoing pregnancy rates leading to live birth of 56%. The mean total number of cycles per patient was 1.8 in the ORT strategy and 1.9 in the SD strategy. The reduction of cycles for the ORT strategy was mainly achieved in the predicted poor responders (1.7 cycles/patient for the ORT strategy vs. 2.1 cycles/patient for the SD strategy). The mean number of 600 IU multidose FSH pens applied per patient was 7.0 in the ORT strategy and 4.8 in the SD strategy. This resulted in an average total cost per patient of €4,149 in the ORT strategy and €4,121 in the SD strategy.

Limitations, reasons for caution: Although we provided ultrasound training and guidelines, no quality control system was operational for AFC-measurements. Despite reported sufficient inter-observer reproducibility, categorization may have been imprecise.

Wider implications of the findings: From a cost-effectiveness perspective, AFC-based dosing has no advantage over standard dosing. However, in the ORT strategy, predicted poor responders underwent less cycles to achieve the same number of live births. In addition, safety aspects related to the prevention of hyperresponse may influence the final valuation of AFC-based dosing.

Trial registration number: Registered at the "Dutch Trial Registry" (www.trialregister.nl). Registration number: NTR2657.

O-038 Testosterone pre-treatment in poor responders treated with GnRH analogues and gonadotrophins for *in vitro* fertilization: a meta-analysis

J. Bosdou[1], E. Kolibianakis[1], C. Venetis[2], T. Tarlatzi[1], K. Chatzimeletiou[1], L. Zepiridis[1], B. Tarlatzis[1]
[1]*Aristotle University of Thessaloniki, 1st Department of Obstetrics and Gynecology, Thessaloniki, Greece*
[2]*University of New South Wales, Dept. of Women's and Children's Health. St George Hospital, School of Women's and Children's Health, Kogarah, Australia*

Study question: Does pre-treatment with testosterone improve the probability of pregnancy in poor responders undergoing ovarian stimulation for *in vitro* fertilization (IVF)?

Summary answer: Testosterone pre-treatment in poor responders undergoing ovarian stimulation for IVF is associated with a significant increase in the probability of clinical pregnancy and live birth.

What is known already: Testosterone pre-treatment has been administered in poor responders undergoing ovarian stimulation for IVF, on the premise that it increases ovarian sensitivity to follicle stimulating hormone (FSH), leading to an improved ovarian response. Several relevant randomized controlled trials (RCTs) have been recently published, evaluating such an intervention without, however, leading to solid conclusions, mainly due to their small sample size.

Study design, size, duration: A literature search was performed until January 2016 aiming to identify RCTs evaluating testosterone pre-treatment in poor responders. Outcome measures included achievement of pregnancy, duration of stimulation, number of cumulus oocyte complexes (COCs), number of 2-pronuclei (2pn) oocytes and number of embryos transferred.

Participants/materials, setting, methods: Five eligible RCTs were identified including 362 patients evaluating testosterone pre-treatment in the form of transdermal (n = 4) or oral (n = 1) testosterone. The dose of transdermal testosterone ranged from 10 mg to 12.5 mg for 15 to 21 days, while testosterone was given per os at a dose of 40 mg/d for 48 days.

Main results and the role of chance: Testosterone pre-treatment was associated with a significantly higher probability of clinical pregnancy (RR: 2.44, 95% CI: 1.47 to 4.03) and live birth (RR: 2.12, 95% CI: 1.20 to 3.76). Moreover, it was associated with significantly lower total dose of gonadotrophins (WMD: −299.73 IU, 95% CI: −552.57 to – 46.89), significantly more COCs (WMD: +0.87 COCs, 95% CI: +0.18 to +1.57) and more 2pn oocytes (WMD: +1.04 2pn oocytes, 95% CI: +0.66 to +1.42). In patients pre-treated with testosterone compared to those who were not, no significant differences were observed in duration of stimulation (WMD: −0.57 days, 95% CI: −1.18 to +0.04, respectively) and in the number of embryos transferred (WMD: +0.15 embryos, 95% CI: −0.06 to +0.36, respectively).

Limitations, reasons for caution: The definition of poor ovarian response as well as the protocols of the proposed interventions and ovarian stimulation protocols varied among studies. Moreover, due to the limited number of studies identified, a meaningful evaluation of clinical heterogeneity was not feasible.

Wider implications of the findings: Currently, based on the limited available evidence, testosterone pre-treatment seems to increase clinical pregnancy and live birth rates in poor responders undergoing ovarian stimulation for IVF.

However, further studies are necessary to increase our confidence to the currently available evidence as well as the precision of the estimates.
Trial registration number: –

INVITED SESSION

SESSION 08: UNDERSTANDING HUMAN REPRODUCTION THROUGH STEM CELLS: TALES OF TRANSLATIONAL DISCOVERIES

Monday 04 July 2016 **Hall 5 CB** **11:45–12:45**

O-039 Naïve pluripotent stem cells: the key to success for *in vitro* gametogenesis?

J. Hanna[1]
[1]*The Weizmann Institute of Science, The Department of Molecular Genetics, Rehovot, Israel*

Abstract text
The identity of somatic and pluripotent cells can be epigenetically reprogrammed and forced to adapt a new functional cell state by different methods and distinct combinations of exogenous factors. The aspiration to utilize such ex vivo reprogrammed pluripotent and somatic cells for therapeutic purposes necessitates understanding of the mechanisms of reprogramming and elucidating the extent of equivalence of the *in vitro* derived cells to their *in vivo* counterparts. In my presentation, I will present my group's recent advances toward understanding these fundamental questions and further detail our ongoing efforts to generate developmentally unrestricted human naive pluripotent cells. I will conclude by highlighting new avenues for utilizing epigenetic reprogramming to naïve pluripotency for unraveling critical gene regulatory mechanisms acting during early mammalian development and highlighting prospects for new platforms for human primordial germ cell and mature gamete developmental modelling.

O-040 Trophoblast stem cells to model earliest steps of placental development

P. Latos[1], A. Murray[1], V. Perez-Garcia[1], C. Senner[1], M. Hemberger[1]
[1]*The Babraham Institute, Epigenetics Programme, Cambridge, UK*

Abstract text
Trophoblast Stem Cells (TSCs) are an invaluable biomedical research tool to study early processes in placental development that are instrumental for pregnancy progression, healthy reproductive outcome and long-term wellbeing. Despite the importance of these processes for normal development, our understanding of the characteristics and requirements of TSCs, and the cues that drive self-renewal and differentiation, remain poorly understood. This is likely the reason why culture conditions for the long-term maintenance of human TSCs have still not been optimized.

Our efforts are focused on gaining a much more refined understanding of murine TSCs so to pave the way towards successful hTSC derivation. Here, we report on our newest insights into the transcriptional and epigenetic regulation of TSCs. We find that their self-renewal capacity is tightly controlled by a stoichiometry-sensitive network made up of the three interacting transcription factors Eomes, Elf5 and Tfap2c. All three factors, when present at approximately equal levels, promote TSC self-renewal. However, a shift in balance towards higher levels of Elf5 and Tfap2c promotes TSC differentiation by re-distributing the chromatin binding sites and activating a differentiation-promoting programme. These findings reveal that the stem cell state of TSCs is exquisitely labile, which may explain the difficulties to maintain their self-renewal potential. At the same time, we are pursuing a CRISPR-Cas9-driven knockout screen in TSCs to determine the origins of developmental abnormalities that may have a placental origin. These efforts demonstrate that our appreciation of the gene complexity required for normal placentation lags far behind that of the embryo itself, and indicates that a significant number of gene mutations causing embryonic defects may indeed be secondary to an abnormal placenta.

INVITED SESSION

SESSION 09: DATA REPORTING SESSION

Monday 04 July 2016 **Hall 5 A** **11:45–12:45**

O-041 Data from the ESHRE PGD Consortium

E. Coonen[1]
[1]*Maastricht University Medical Center, Obstetrics and Gynecology, the Netherlands on behalf of the PGD Consortium*

INVITED SESSION

SESSION 10: THE EARLY EMBRYO - GENETICS AND DEVELOPMENT

Monday 04 July 2016 **Hall 3 AB** **11:45–12:45**

O-042 FSA exchange lecture: Validation of next-generation sequencing for testing chromosome aneuploidy in preimplantation embryos

P. Coleman[1], M. MARTIC[1], S. STOCK-MYER[1], L. WILTON[1],
[1]*Melbourne IVF, Preimplantation Genetics, Melbourne, Australia*

Abstract text
Aim: A variety of techniques for 24-chromosome analysis are currently available for clinical use in preimplantation genetic diagnosis for aneuploidy PGD-A. ArrayCGH (comparative genomic hybridisation) was the first widely available technology used for aneuploidy testing on embryos in PGD programs. Next generation sequencing (NGS) based aneuploidy testing has recently been introduced. Here we present the application of an NGS based chromosome testing system (Illumina, USA) on single cells and trophectoderm (TE) samples.
Method: Aliquots of whole genome amplified (Sureplex, Illumina, USA) DNA from either single cells or TE samples that had been previously analysed using arrayCGH were coded and subjected to NGS. The chromosome status of these samples was analysed with no knowledge of the previous arrayCGH result. Data was then unblinded and concordance determined.
Results: Amplified products from 170 single blastomeres and 8 TE samples were analysed by NGS. Results were obtained on 100% of amplified products. In each case the result obtained by NGS confirmed the clinical result diagnosed by array CGH (sample concordance 100%). Individual chromosome concordance was 99.97% (4271/4272).
Conclusion: This study has demonstrated that NGS can provide 24-chromosome aneuploidy results for single cells and TE samples with a high level of consistency to established array CGH methodologies. Our PGD laboratory is now participating in a world-wide randomised clinical trial to validate the clinical effectiveness of NGS technology.

As a result of our validation of this system we are now using NGS clinically for aneuploidy diagnosis and have processed and reported over 1000 samples.

O-043 Getting turned on after sex: the three first days of the embryo

INVITED SESSION

SESSION 11: PARAMEDICAL INVITED SESSION - LABORATORY

Monday 04 July 2016 **Room 101** **11:45–12:45**

O-044 Oocyte on crutches

C. Andersen Yding[1]
[1]*Copenhagen University Hospital Rigshospitalet, Section 5712- Laboratory of Reproductive Biology, Copenhagen, Denmark*

Abstract text

In the early/mid-1990s ICSI was developed for severe male infertility and enabled men to become farther with few or immotile sperm cells by facilitating fertilization of oocytes. Now the time has come for female counter part, the oocyte, to undergo development enhancing measures to help women who would otherwise have difficulties in becoming mothers. The mechanisms that cause oocytes to have difficulties to sustain fertilization and further development are now slowly being unraveled. Alongside comes new potential methods that may be employed to overcome these difficulties. Together with these very sophisticated ways of manipulating oocytes and indeed cellular components of oocytes or other cells have allowed development of experimental methods, which we have only seen the very first start of.

A major cause for the development of these new techniques is the fact that women wait too long to have children. The quality of the oocytes becomes reduced with age and one the main challenges for today's fertility clinics are reproductive aged women, who in their late thirties or later wants to conceive. Such women often either produce only a limited amount of oocytes and/or the quality is reduced to a limit where pregnancy becomes a difficulty.

One method that has attracted a lot of interest is a direct invasive method that aims to improve the quality of the oocyte. The energy consumption used to sustain fertilization is considerable and in some women's oocytes the cellular energy producer, the mitochondria, is not working optimally. A new method is now basically attempting to recharge the oocyte's energy producing capacity by injecting the woman's own mitochondria. Mitochondria contain their own DNA and are believed to undergo mutations more often than normal cellular DNA. In order to circumvent this problem the first attempt to use technique has employed a technique in which oogonial stem cells from a piece of ovarian tissue is produced from the patient herself. Only from oogonial stem cells, once established as a cell line, are the mitochondria purified and used to inject into the oocytes. Only preliminary clinical data have yet emerged and potential positive effect remains to be determined. It is now discussed whether the mitochondria actually needs to be derived from oogonial stem cells or may be derived from for instance granulosa cells, immature oocytes or preantral follicles from the same woman. Furthermore, mitochondria may be found in an activated form ready to produce energy and a more latent form in which energy production is less efficient, and it appears that only a small part of the oocytes mitochondria are active. Therefore, it may only necessary to inject relatively few activated mitochondria to enhance the energy production from the oocytes. The coming year's research will undoubtedly put light on these issues.

The lecture will in addition cover other aspects of performing oocyte performance enhancing measures to improve fertilizing capacity and the enable embryo production.

O-045 Non-invasive markers for embryo selection

H.N. Sallam[1]

[1]*Alexandria University Faculty of Medicine, Department of Obstetrics and Gynaecology, Alexandria, Egypt*

Abstract text

There exists a need for a reliable non-invasive method for embryo selection in women undergoing IVF to maximize the chances of a live birth and minimize the incidence of multiple pregnancies and its complications. Many methods have been suggested and practiced and the aim of this presentation is to evaluate these methods in the light of evidence. Proper evaluation requires constructing a receiver operating characteristic (ROC) curve followed by a randomized trial (RCT) in single embryo transfer cycles.

Since the beginning of IVF, embryos were selected on the basis of their morphology at the pronuclear stage (Scott et al., 2000), the cleavage stage (Giorgetti et al., 1995; van Royen et al., 1999; Hsu et al., 1999; Houghton et al., 2002) or the blastocyst stage (Gardner et al., 2000). Attempts at giving more subjectivity to morphological selection by calculating a graduated embryo score (Fisch et al., 2001) or by the computerized transformation of the embryo picture into a mathematical vector have not shown their superiority (Manna et al., 2004). Embryo selection by natural depletion in women with a large number of embryos has also been practiced and a meta-analyses of RCTs show that the transfer of embryos at the blastocyst stage is associated with a small but significantly higher live birth rate compared to cleavage stage transfers (Glujovsky et al., 2012).

A more recent approach is embryo selection on the basis of morpho-kinetics studied by time lapse photography (TLP) and the constructions of algorithms based on defined kinetic and morphologic markers. Despite the early optimism, a recent Cochrane review found insufficient evidence of differences in live birth or clinical pregnancy between TLP and conventional incubation (Armstrong et al., 2015) and a recent RCT concluded that embryo selection by TLP did not significantly improve clinical reproductive outcomes (Goodman et al., 2016). Embryo selection on the basis of oxygen consumption (OC) by embryos has also been tried, but a prospective RCT is still needed to confirm the validity of this method (Tejera et al., 2012).

Biochemical markers have also been studied in the spent culture medium in an attempt to select the embryo(s) with the best potential for implantation. These include pyruvate and glucose uptake as well as lactate production (Gott et al., 1990) and recently Gardner et al found that glucose uptake on day 4 of human embryonic development was significantly higher in embryos resulting in pregnancy (and in those resulting in female offspring) (Gardner et al., 2011). Similarly, Houghton et al studied the amino acids turn-over rate in the spent culture medium of embryos and found different patterns in embryos achieving pregnancy compared to those that did not (Houghton et al., 2002). Measurement of embryo metabolomics by spectroscopic analysis (Seli et al., 2010) as well as by nuclear magnetic resonance (Kirkegaard et al., 2014) has also been tried. However, a meta-analysis of 4 prospective randomized trials found no evidence that near infra-red (NIR) spectroscopy of spent embryo culture media improves live birth rates (Vergouw et al., 2014). Soluble human leukocytic antigen G (s-HLA-G) fragment was also studied and found to be a good predictor of embryo viability (Vercammen et al., 2008). However, prospective RCTs are still needed to confirm the validity of this method. Other biochemical markers include β-HCG, haptoglobin-α-1 fragment and platelet activating factor (PAF) (Xiao-Yan et al., 2013; Montskó et al., 2015; Roudebush et al., 2002). Finally, embryo selection by measurement of oxidative stress has been tried with promising results but prospective RCTs are still awaited (Wiener-Megnazi et al., 2011).

In summary, morphology assessment by an experienced embryologist remains the best available method for embryo selection until otherwise proved.

INVITED SESSION

SESSION 12: OPTIMISATION OF THE MONITORING OF OVARIAN STIMULATION

Monday 04 July 2016 **Hall 5 CB** **14:00–15:00**

O-046 The value of tele-monitoring in IVF/ICSI

J. Gerris[1]

[1]*University Hospital Ghent, Centre for Reproductive Medicine – Deptartment of Ob/Gyn, Gent, Belgium*

Abstract text

The need for serial vaginal sonograms to monitor ovarian stimulation for artificial reproductive technology (ART) treatments remains a cause for stress and sometimes a major practical challenge both for patients and health care providers. It hampers access to treatment for many couples seeking ART treatment or renders it strenuous from an organizational point of view. It creates a hidden cost when people have to travel frequently to have sonograms made or have to plan a hotel stay near the centre where oocyte retrieval and IVF embryology work take place. We have been exploring over the past couple of years the possibilities of a method (Self-Operated Endo-vaginal Tele-monitoring, SOET) allowing patients and/or their partners to make vaginal sonograms themselves at home or in fact anywhere and anytime if WiFi is available. Specific cloud-based communication software has been developed that can be installed on any notebook or tablet PC. If applicable, midwives, nurses or other health care providers such as general practitioners working independently from highly specialized services, can also perform these recordings. Current experience includes results from a published randomized trial (Hum. Reprod. 2014) comparing SOET with traditional monitoring as well as from a subsequent series of one hundred consecutive ART cycles where patients used SOET instead of traditional monitoring to monitor the stimulation phase. Clinical, laboratory and patient reported outcome data as well as health economic data show that after some initial teaching motivated patients are sufficiently prepared and competent to make and send adequate video recordings to the centre or to the physician following them up. The interpretation of these recordings does not lead to different

clinical decisions than when made on-site using high-end machines. On-site back-up remains always possible if there is a need to confirm unexpected results. This was the case is just 1% of patients. Laboratory and clinical results are similar to those obtained with traditional monitoring and patients are very satisfied with SOET. They can make sonograms discretely where and when it suits them best. Receipt of images and of responses is acknowledged by SMS. After measurement of the growing follicles, a structured response is sent to the patient, comprising dosing advice and next-step instructions. This facilitates the follow-up of ovarian stimulation, even if applicable initially to just a selected proportion of IVF patients. It can fit into the general tendency to make IVF more patient-centred and -friendly, to implement tele-medicine and increase patient empowerment and partner participation by supervised active participation to their treatment. It opens up access to treatment for patients who live far from IVF centres and widens the scope of patient recruitment for centers.

If combined with point of care determinations of estradiol to monitor patients at risk for OHSS, SOET may acquire a place in ART although the most recent Cochrane meta-analysis concludes that systematic E2 measurements do not increase pregnancy rates nor decrease the incidence of OHSS.

Impediments for implementation of this technology are disruption of the comfort zone of doctors, the novelty of the technology and financial considerations. SOET is not just a tool, but a disruptive change of practice where the patient and her partner are playing a central and active role in their treatment.

O-047 Optimising IVF outcome by monitoring serum progesterone levels during ovarian stimulation

C. Venetis[1]
[1]*University of New South Wales, UNSW Medicine, Sydney, Australia*

Abstract text
The prognostic role of progesterone elevation (PE) for the outcome of a fresh IVF cycle has been debated for more than 20 years. Following the publication of a meta-analysis of more than 60,000 cycles, there is now unequivocal evidence that in the presence of elevated progesterone on the day of hCG administration for final oocyte maturation the probability of pregnancy after a fresh embryo transfer is significantly reduced. This effect, believed to be exerted *via* the action of progesterone on the endometrium, is not negligible, since in certain cases it can be translated to a relative reduction of 30% or more in the effectiveness of an IVF cycle.

Not surprisingly, this has ignited a discussion among clinicians and clinical researchers on what is the most efficient way serum progesterone levels can be incorporated in routine monitoring in order to optimize IVF outcome.

Firstly, it has been shown that the cut-off of PE on the day of hCG might be not be the same for each clinic. This is highly dependent on the assay and the individual characteristics of the population treated in each setting. In order to identify the net effect of PE on the treated population of a specific IVF clinic, a rigorous methodological approach has to be applied with the removal of the potential confounding effect of a range of variables, including female age, the number of oocytes retrieved and the quality of the embryos transferred.

Some authors have also tried to identify a potentially better prognostic tool for IVF cycles that incorporates the serum concentration of progesterone. The measurement of progesterone on consecutive days during the late follicular phase and the calculation of the relevant area under the curve, as well as the ratio of progesterone-to-estradiol or progesterone-to-follicle have been proposed. However, there is no convincing evidence that these convey any benefit when compared to a single progesterone measurement on the day of hCG and, in certain cases, their physiological basis is questionable. The answer might actually be a more straightforward and physiologically sound approach which involves the incorporation of the serum progesterone concentration on the day of hCG in a prognostic model for live birth after IVF.

Two main strategies have been proposed in order to counteract the detrimental effect of elevated progesterone on pregnancy rates after a fresh embryo transfer. Prevention of the occurrence of PE and freezing-all embryos and deferring transfer in a frozen–thawed embryo transfer cycle.

Since PE on the day of hCG is known to be strongly associated with the magnitude of ovarian response, mild ovarian stimulation has been suggested as a method to prevent its occurrence. Identifying patients at high risk for PE is a key component of this strategy. Apart from known risk factors of high ovarian response, basal progesterone and previous history of PE have been shown to be strong predictors of PE on the day of hCG after ovarian stimulation for IVF.

Finally, in the presence of elevated progesterone on the day of hCG, elective cryopreservation of all embryos and subsequent transfer in a frozen–thawed embryo transfer cycle appears to be the most popular strategy, although high quality evidence originating from large RCTs is urgently warranted, especially for patients with a small number of oocytes retrieved.

INVITED SESSION

SESSION 13: CELLULAR INTERACTIONS IN OOCYTE PHYSIOLOGY

Monday 04 July 2016 **Hall 5 A** **14:00–15:00**

O-048 Dynamic imaging of the oocyte-granulosa dialogue

D. Albertini[1]
[1]*The Center for Human Reproduction, New York, NY, USA*

Abstract text
The ovarian follicle exploits the properties of a true cellular syncytium to maintain coordination of the processes of oogenesis and folliculogenesis. For over 30 years now, it has been appreciated that the syncytial properties of the follicle are dependent upon specialized intercellular junctions such as gap junctions mediating information exchange between granulosa cells and between granulosa cells and the oocyte. This presentation will review recent data on the role of specialized extensions from cumulus cells, known as transzonal projections (TZPs), in supporting the growth and maturation stages of oogenesis as revealed by the use of high resolution imaging modalities. TZPs are dynamic structures penetrating the zona and establishing specialized contacts with the oolemma in response to oocyte secreted factors during the growth stage of oogenesis and are negatively regulated by pulsatile FSH signals to establish the cumulus and mural cell lineages. The maintenance of a committed cumulus is required for the continued metabolic cooperation between somatic and germ cell compartments that includes provision of cholesterol synthesized in the cumulus and directly transferred to the oocyte *via* terminations of the oocyte that are both rich in mitochondria as well as vesicular exchange. The complexity of mechanisms available for intercellular molecular exchange at the cumulus-oocyte interface assures integration of the developing oocyte with that of its companion follicle and is likely at the heart of variations in oocyte and embryo quality observed with advanced maternal age.

O-049 RNA accumulation in competent oocytes

M.A. Sirard[1], A. Macaulay[1], R. Labrecque[1], E. Orozco-Lucero[1], D. Khan[1], C. Robert[1]
[1]*Université Laval, Deptartment of Animal Sciences, Quebec, QC, Canada*

Abstract text
The oocyte is invested with the responsibility of providing the infrastructure and the template for early embryonic development. The growth period must therefore prepare the oocyte for functions that are not required immediately but will be activated following the shutdown of transcription which occurs a few days prior to ovulation in most mammalian species. What can be stored in the form of proteins is already present in fully grown oocytes like cortical granules, most of the cytoskeleton and all organelles. In fact, the protein profile for the most abundant (~2000) proteins changes very slightly during that period but the mRNA profile and/or the de novo protein synthesis changes rapidly due to RNA accumulation and their specific recruitment. In larger mammals, unlike the mouse, the capacity of the oocyte to sustain embryonic development is not fully functional when the oocyte is fully grown but is acquired progressively as the follicles reaches dominance and it exhibits LH receptors. Therefore, the final accumulation of mRNA is critical during late folliculogenesis and specific follicular (granulosa cells) transcripts have now been associated with better developmental potential of the oocyte. In our bovine model, oocytes of different qualities can be generated by stimulating the development of dominant follicles with exogenous FSH and then depriving these follicles from FSH support for 1–2–3 or 4 days in the presence of low LH and high progesterone. This experiment increases oocyte quality as measured by blastocyst rates after IVF to an average of 75% in first 2 days followed by a decrease in quality at 4 days.

We have analyzed the RNA content by microarray and observed that transcripts associated with the control of mRNA processing are essential to support the embryonic program to follow. These mRNA can be followed through polyadenylation analysis, polysomal extraction and protein translation to identify those transcripts that are required during maturation, fertilization or early embryonic development. Although the source of these RNAs is believed to be principally from the oocyte nuclear transcription, recent evidences demonstrate that some RNA could be transmitted though trans-zonal projections. These RNAs would be synthesized in the cumulus, transported along the trans-zona projections and secreted as exosomes in the perivitellin space. This process was demonstrated using ultrastructure microscopy, immunohistochemistry, transfection analysis and genomic analysis. The oocytes show a rise in several transcripts in the last 2 days before ovulation and could be divided into two groups: the ones that may be used immediately and are present in the polysomes at metaphase II and the ones that have short poly-A tails and are present in higher quantity in early cleaving two-cells embryos, a sign of their implication in higher competence. The combination of several data sets now allow the identification of specific targets such as ATF1 that could be the result of final follicular signaling to improve oocyte quality before ovulation. In conclusion we believe that the follicle-cumulus-oocyte syncytium works as a single unit to ensure the proper accumulation of embryo-permissive mRNAs in the oocyte.

INVITED SESSION

SESSION 14: WHAT IS THE PROBLEM WITH ANONYMITY IN DONOR CONCEPTION?

Monday 04 July 2016 **Hall 3 AB** **14:00–15:00**

O-050 Anonymity in donor conception should not be allowed

P. Thorn [1]
[1]*Mörfelden, Germany*

Abstract text

In the context of donor conception, the term "anonymity" refers to the semen or egg donor/s being "nameless" for the intended parents and the child. Until the 1990s, anonymity as well as secrecy were common: It was feared that children may be confused about the "real" parent, bonding between the child and the social parent was assumed to be less secure if the child was aware of his/her conception, donors were not protected from legal paternity and medical professionals feared stigmatization if it became known that they carried out treatment socially unaccepted.

This has changed in the last two decades. In several countries, legislation has granted offspring the right to access the donor's identity and exempted the donor of any parental rights or responsibilities. Although there is no representative data, there is anecdotal evidence that an increasing number of parents share the donor conception with their child and significant others. Furthermore, more and more offspring are interested in securing information about their biological origins and indicate curiosity about half-siblings.

At the same time, the debate surrounding anonymous and identifiable donors remains controversial. Although sharing the conception with children even in countries with donor anonymity has become less exceptional, legislation itself in these countries remains unchallenged. Donors' identity remains shielded by legislation, as there are not only concerns that the number of donors may decline significantly if anonymity was abolished but also that family harmony may be strained if children knew their donor. In other countries, legislators send contradictory messages and add to ambiguities by granting offspring the right to access their biological origin but not protecting donors sufficiently from legal responsibilities. This suggests that some previous anxieties persist and that the stigma surrounding these (relatively) "new" family compositions continues.

In order to avoid frustration for offspring and provide them with equal rights as adoptees, it is vital to discontinue anonymity. Donor anonymity results in depriving offspring information about their biological heritage, which is taken for granted by many people in most cultures. Instead of minimizing the role of the donor, anonymity can have the adverse effect: Both parents and children may project unrealistic fantasies upon the donor and are unable to adjust them to reality. Current practice and anonymity also limits the information donors receive about the outcome of their donation and only little is known about their needs. However, there is an imbalance between the adult parties who can consent into an anonymous donation and the child who cannot. As long as there is the probability that children feel thwarted by anonymity, feel underprivileged in comparison to children in other family types and face the risk of an identity crisis, legislators have the duty to introduce legislation that avoids potential harm from these children.

However, legislating against anonymity is not sufficient. Infertility and donor conception are associated with deep-rooted stigmata which can only be tackled if a multi-level approach is applied: On the individual level, intended parents and donors should be recommended pre-treatment/pre-donation counselling in order to minimize anxieties and increase confidence; on the professional level, a framework for counselling imbedded in medical treatment and on-going multi-professional collaboration is needed; on the macro level legislative frameworks that protect the donor from responsibilities, that grant parents unambiguous legal maternity/paternity and offspring the right to access their biological origins as well as the possibility to contact half-siblings have to be introduced. If efforts are directed effectively at all three levels, the stigma will decrease, the dominant pattern of anonymity will change and donor anonymity will not be necessary anymore.

O-051 Anonymity in donor conception should be a parental choice

W.J. Dondorp[1]
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In this debate session I will defend that prospective parents should be allowed to choose between anonymous and open-identity gamete donation and that this approach best serves the interests of all parties concerned. I will argue that there are no good reasons for the mandatory anonymity that existed in the past. As there is no evidence that open-identity gamete donation entails a high risk of serious harm for the child, it is in line with the "welfare of the child" document of ESHRE's Task Force Ethics and Law (2007) to allow this as a parental choice. Many recipients of donor gametes find it important for their future child to have the opportunity to know the identity of the donor and perhaps also have contact with him or her. But similarly, there are no convincing arguments for disallowing the opposite. As there is no evidence that anonymity in donor conception entails a high risk of serious harm for the child, this should also be an option, available to recipients who have different views of what would best serve the interests of their child and their family. Either way, this should be respected as belonging to the sphere of parental responsibility. The tendency in several countries to move from mandatory anonymity to imposed non-anonymity is ethically problematic, as both positions limit reproductive freedom for no good reason. For some of those who are in need of donor gametes for reproduction open-identity donation is simply not an acceptable option. They may have difficulties accepting a potential role of the donor as a third person in the future life of their family, or have no intention to disclose the method of conception to their child (for instance because donor conception is not well accepted in their community). Imposed non-anonymity may mean that these people will see no other option but to go to countries with different legislation, or if that proves to costly, may have to refrain from having children altogether. This is a high price to pay, that would be justified if it could be shown that anonymous donation leads to serious harm for donor children. But that, I argue, is not the case.

SELECTED ORAL COMMUNICATIONS

SESSION 15: PARAMEDICAL 1 - NURSING

Monday 04 July 2016 **Room 101** **14:00–15:00**

O-052 Quality of life and predictive factors in a French cohort of infertile women undergoing assisted reproductive technology

J. Gonnot[1], M. Bourdon[1], V. Blanchet de Mouzon[1], C. Cervantes[1], V. Gayet[1], J. De Mouzon[2], P. Santulli[1], D. De Ziegler[1], C. Chapron[3]
[1]*Hopital Cochin Port Royal, Service Médecine de la Reproduction, Paris Cedex 14, France*

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[3]Hopital Cochin Port Royal, Service de Gynécologie Obstétrique II et Médecine de la Reproduction, Paris Cedex 14, France

Study question: To evaluate quality of life (QOL) in a cohort of women undergoing assisted reproductive technology (ART) and to identify predictive factors of poor QOL.
Summary answer: In French women, the risk of poor QOL is significantly increased with shorter infertility duration.
What is known already: Many published studies reported that assisted reproductive technology (ART) could be stressful and affect QOL in infertile women. Boivin et al. (2011) developed the fertility quality of life (FertiQol) score. FertiQol items were designed to translate QoL into quantitative items that could collectively indicate the impact of fertility problems. To date, no study has been performed in a large French cohort of infertile women requiring ART.
Study design, size, duration: Retrospective observational cohort study conducted between 01/01/2014 and 31/06/2014 in a tertiary care university hospital. A total of 166 patients who underwent IVF or ICSI programs were analysed. QOL was assessed using FertiQol International evaluation before starting IVF treatment. The evaluation consists of two validated modules measuring QoL (the FertiQoL Core and the Optional FertiQoL Treatment module). We defined poor QOL as a Total FertiQol score below the 25th percentile.
Participants/materials, setting, methods: A total of 166 patients completed the self-administered FertiQol questionnaire. Women were allocated to two groups according to the situation of their total FertiQol score towards the 25th percentile: ≤25th (n = 40) or >25th percentile (n = 126). Statistical analysis was conducted using univariate methods and multivariate logistic regression models. Statistical significance was obtained for $p < 0.05$. In the logistic model, results were given as odds ratios (OR) with their 95% confidence interval (CI).
Main results and the role of chance: Median Total FertiQol score in our population was 65.44/100. The 25th percentile was 57.35/100. At univariate analysis, women with poor Qol (total FertiQol score ≤25th percentile) were significantly more often aged less than 38 years compared to the group of QoL higher than the 25th percentile (82.5 vs. 62.7%, $p = 0.02$). Similarly, an infertility duration of less than 5 years was more often observed (82.5 vs. 62.0%, $p = 0.02$). After multivariate logistic regression, only infertility duration of less than 5 years was associated with a Total FertiQol Score below the 25th percentile (OR = 3.89, 95% CI: 1.26–12.01, $p = 0.02$).
Limitations, reasons for caution: Young infertile women discover the difficulties of ART treatment, while older women, who have a long medical history of infertility, seemed to be more familiarized by care pathways, which could causes interferences between the answers.
Wider implications of the findings: Women with short duration of infertility were associated with worse QoL. These findings can be used to achieve a higher quality of care for patients, in order to increase their QoL. This may be added to the recent ESHRE guidelines on psychological counselling, according to women characteristics, published in 2015.
Trial registration number: Not applicable.

O-053 Fertility preservation brings hopefulness and wellbeing for young oncologic women

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Study question: What are the feelings, worries, life's quality impact about fertility preservation in oncological patients?
Summary answer: Desire of motherhood is intrinsic to women, but cancers bring helplessness. Fertility-preservation brings hopefulness and safety; women would be disturbed if it wouldn't be performed.
What is known already: Infertility is a major consequence of cancer treatment. In young women the risk of ovarian failure after radiotherapy and/or chemotherapy is of concern, as it can reach up to 80% of cases. Thus, the worry about reproductive future of those patients deserves increased attention. There is no doubt about the psycho-emotional gains of fertility preservation after cancer diagnosis, as the impossibility of biological conception is one of reasons for anxiety and can affect even the cancer recovery. In addition, FP for cancer women can positively influence a patient's overall wellbeing by lessening anxiety due the chance fertility loss.
Study design, size, duration: This is a qualitative cross-sectional study based on a questionnaire applied to a selected group of women diagnosed of cancer, who underwent fertility-preservation at least 6 months before the study (November–December, 2015). Twenty-three eligible women were contacted by phone and the questionnaire was applied and completed in 16 women. Seven women were to follow-up. The structured questionnaire contained 16 questions, 14 multiple-choice and 2 opened questions.
Participants/materials, setting, methods: The questions enquired about actual cancer conditions, decision-making process of fertility-preservation, feelings at fertility-preservation moment, actual feelings, possibility of using the oocytes/embryos cryopreserved, importance and impact of fertility-preservation in their life. Participants could choice more than one option for the multiple-choice questions. We excluded one teenager and 15 women ageing 23–39 years were included in the study.
Main results and the role of chance: Among women interviewed, 10 became disease-free and 5 are under cancer treatment. At the moment of fertility-preservation, no patient had a child, and most of them (73%) declared wish of having children. When they were argued about feelings at the fertility-preservation time, the most frequent answers were hope (26.3%), safety (26.3%), relief (26.3%) and peacefulness (15.8%). One patient (5.3%) reported suffering. Regards the decision about fertility-preservation, time (38.8%) was the main challenging point, followed by financial costs (33.3%). At the interview, all women declared the importance of fertility-preservation; 73.4% stated that it is warrant the possibility of a biological pregnancy, due to risk of infertility after chemio-radiotherapy. Finally, women were argued "if they had not done the fertility-preservation, how would they feel and what would be the impact in their lives?" The answers stated emotional impairment, low quality of life, relationship problem and uncertainty about the maternity.
Limitations, reasons for caution: The number of women interviewed is a limitation. However, those are preliminary results and we are continuing the interviews when women had completed 6 months after fertility-preservation. Also, opened questions make interpretation difficult, but an appropriated analysis method will be used when a higher number of patients are included.
Wider implications of the findings: Fertility-preservation strategy for oncologic patients is a positive approach, as it increases the chance of biological child in the future. Beyond this, all women felt the fertility-preservation is a worth process and the safety of had done FP brings peacefully for the oncological treatment and higher quality of life.
Trial registration number: Not applicable.

O-054 The association between endometriosis patients' quality of life and the patient-centeredness of their care

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Study question: Is quality of life associated with patient-centered care in women with endometriosis?
Summary answer: There was a trend towards a significant association between the quality of life of patients with endometriosis and the experienced patient-centeredness of their care.
What is known already: The physical symptoms of endometriosis can have a negative impact on women's quality of life. Qualitative studies suggest that endometriosis patients' quality of life is associated with the patient-centeredness of their health care. Quantitative studies showed a significant association between quality of life and patient-centered care in subfertile women but not yet in women with endometriosis.
Study design, size, duration: For this cross-sectional study, valid and reliable questionnaires were administered by postal mail in 2011. Non-responders received two reminders. In total, 109 out of 194 eligible patients responded (56%).

Participants/materials, setting, methods: Dutch speaking women having been diagnosed with endometriosis and treated by laparoscopy for pain and/or infertility in a Dutch tertiary fertility clinic between 2009 and 2010 were eligible. Quality of life was assessed with the Endometriosis Health Profile-30 (EHP-30) and patient-centeredness with the ENDOCARE-questionnaire (ECQ). For both measures scores were converted to a scale from 0 to 100. Higher scores reflected worse quality of life and less positive healthcare experiences. Linear regression analyses were conducted.
Main results and the role of chance: Participants had a mean age of 35 years. The majority self reported a diagnosis of moderate to severe endometriosis (79%) and experienced endometriosis-related symptoms during the past year (89%). The mean overall quality of life was 29/100 and the mean overall experience score for patient-centeredness was 38/100. Regression analyses found no significant associations between overall quality of life and any of the ten dimensions of patient-centered care after controlling for experiencing endometriosis-related symptoms during the past year. A trend towards a significant association between overall quality of life and the dimensions "respect for patients" values, preferences and expressed needs' (B = 0.19; p = 0.103) and "information, communication and education" (B = 0.26; p = 0.095) was observed. The "emotional well-being" dimension of quality of life was associated with the patient-centered care dimensions "information, communication and education" (B = 0.33; p = 0.046) and "respect for patients" values, preferences and expressed needs' (B = 0.29; p = 0.023). Additionally, the "social support" dimension of quality of life was also associated with the patient-centered care dimension "information, communication and education" (B = 0.42; p = 0.023).
Limitations, reasons for caution: The cross-sectional design of this study does not allow drawing conclusions on the direction of associations. The single-center setting resulted in a small sample size. This could, in turn, explain why no significant associations were found between overall quality of life and patient-centeredness.
Wider implications of the findings: Providing patient-centered care might lead to improved quality of life in women with endometriosis, especially if paying attention to the dimensions "respect for patients' values, preferences and expressed needs" and "information, communication and education." Large-scale longitudinal research is needed to investigate this further.
Trial registration number: NA.

O-055 Psychosocial vulnerability identified by screening in early pregnancy is not increased after fertility treatment.

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Study question: Is psychosocial vulnerability in early pregnancy increased after fertility treatment?
Summary answer: After early antenatal screening for psychosocial vulnerability similar risk prevalence was found among women who conceived with or without fertility treatment, but psychosocial-diagnosis differed.
What is known already: More than 10% of mothers develop postpartum depression (PPD), and additionally 5–10% show other mental reactions such as anxiety. In order to cope with psychosocial problems during pregnancy and postpartum, Danish pregnant women are classified using a risk score of 1–4, recommended by the National Board of Health. Women at risk, are offered additional care in an expanded antenatal care unit. According to Danish Law, if medical professionals before initiating fertility treatment suspect parenthood unsuitability, the patients should be referred to the State Administration in order to decide whether treatment can be offered or not.
Study design, size, duration: This cross-sectional study includes all pregnant women referred to the antenatal clinic, at Copenhagen University Hospital Rigshospitalet, through 2013–2015. At the antenatal clinic, a screening-program for psychosocial risk factors is carried out. The screening-program consists of a questionnaire. If the woman shows parameters of risk, follow-up by telephone-interview is made. Hereafter it is decided whether the patient should be referred to the Expanded Antenatal Care-Unit (EAC-Unit), a specialist-team of doctors, nurses, midwives, psychologists and social-workers.
Participants/materials, setting, methods: All patients (n = 18.038) referred to the antenatal clinic were classified according to methods of conception; i.e., conceived after fertility treatment (study group) or naturally conceived (control group). The prevalence of women referred to the EAC-Unit was calculated for the two groups and the psychosocial diagnoses were compared. The following diagnoses were recorded: depression, anxiety, eating disorders, other psychiatric diagnoses, various social problems, history of abuse, abuse in pregnancy, repeated miscarriages and previous stillbirth.
Main results and the role of chance: In total 1,877 (10.4%) of all the women referred to the antenatal clinic were pregnant after fertility treatments and of these 154 (8.2%) were referred to the EAC-Unit. In the control group of natural conceptions (n = 16.161; 89.6%) a similar proportion of pregnant women (1,519/16,161; 9.4%) were referred to the EAC-Unit. The following psychosocial diagnoses were recorded among the fertility patients versus the control group: (1) Depression and/or anxiety: 43/154 (27.9%) versus 470/1,519 (30.9%) (p = 0.44); (2) Eating disorders: 16/154 (10.4%) versus 154/1,519 (10.1%) (p = 0.92); (3) Other psychiatric diagnosis 10/154 (6.5%) versus 216/1,519 (14.2%) ($p \leq 0.05$); (4) Social problem 14/154 (9.1%) versus 257/1,519 (16.9%) ($p \leq 0.05$); (5) History of abuse 2/154 (1.3%) versus 46/1,519; (3.0%) (p = 0.22); (6) Abuse in pregnancy 0/154 (0%) versus 50/1,519 (3.3) ($p \leq 0.05$); (7) Repeated miscarriages 3/154 (1.9%) versus 20/1,519 (1.3%) (p = 0.54); (8) Previous stillbirth 9/154 (5.8%) versus 58/1,519 (3.8%) (p = 0.22); (9) Other problems/unclassified 57/154 (37.0%) versus 248/1,519 (16.3%) ($p < 0.01$).
Limitations, reasons for caution: Data were collected from a tertiary referral hospital, placed in the capital of Denmark, which includes highly specialized functions. The consequence for our investigation could be an increased prevalence of patients with psychosocial vulnerability, but this would influence the results in both groups.
Wider implications of the findings: Our results reflect the intention of the Danish law on ART that patients treated should be similar in terms of psychosocial characteristics compared to the general population. It is reassuring that the proportion of ART patients considered to need special psychosocial care did not differ from the background population.
Trial registration number: No trial number.

SELECTED ORAL COMMUNICATIONS

SESSION 16: PREIMPLANTATION GENETIC SCREENING AND MITOCHONDRIAL DNA

Monday 04 July 2016 **Hall 1** **15:15–16:30**

O-056 Day 7 blastocysts prove beneficial for preimplantation genetic screening cycles.

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Study question: Are there benefits to culturing embryos 168 h post-insemination? Do day 7 blastocysts result in similar aneuploidy and implantation rates compared to day 5/6?
Summary answer: Culturing embryos to day 7 has proven beneficial to achieving viable euploid blastocysts. Day 7 growth is poor but euploidy is similar to day 5/6.
What is known already: Routine human blastocyst culture extends embryo development up to 6 days or 144 h post-insemination. *In vivo* embryo implantation is theorized to initiate sometime from 96 to 144 h post-insemination, but exact limits and timing are relatively unknown. Furthermore, it is hypothesized that *in vitro* cultured embryos may be delayed in their development compared to their *in vivo* counterparts. For many programs, embryos must display viability prior to transfer and/or cryopreservation, with subjective evaluations concluding on the sixth day of culture. With an embryos' ploidy status influencing implantation, it is unknown if prolonged embryo culture increases aneuploidy.
Study design, size, duration: A single center prospective observational cohort study analyzing 292 autologous oocyte cycles (average age: 37.3 ± 4.3) and 23 donor oocyte cycles yielded 1,565 fair-to-excellent quality blastocysts (i.e., BB to AA, respectively), between January 1, 2015 and December 31, 2015. These patients were enrolled in *in vitro* fertilization treatments involving blastocyst

biopsy/cryo-all cycles. All cycles included PGS with trophectoderm biopsy for aneuploidy determination. Only vitrified-warmed single euploid blastocyst transfers were performed. Differences were assessed by Chi-squared analysis.
Participants/materials, setting, methods: Patient embryos were cultured in Life Global media with LGPS under tri-gas incubation conditions, and biopsied at or beyond the full blastocyst stage. Embryos grown to day 7 failed to meet quality or expansion criteria to biopsy by day 6. All trophectoderm biopsy samples were analyzed using NextGen sequencing. These cycles resulted in 151 single embryo transfers; 87 using a day 5 blastocyst, 53 day 6 blastocysts and 11 day 7 blastocysts.
Main results and the role of chance: Day 7 blastocysts achieved a euploidy rate of 36% (n = 111) per biopsy with top quality day 7 blastocysts (i.e., AA, AB, BA) achieving 50% euploidy (n = 50). Although blastocyst formation on day 7 was poor and only accounted for 6.3% of all blastocyst development, the resulting transfer of euploid blastocysts (n = 11) achieved a 63.6% implantation, which was not statistically different than day 5 or day 6. Interestingly, there was an increased loss rate of 28.5% with the day 7 blastocysts. The ongoing pregnancy rate of 45.5% was lower ($p < 0.05$) than both the day 5 (75.9%) and day 6 (69.8%) embryos. There were no differences observed for implantation, embryonic/fetal loss or ongoing pregnancies between day 5 and day 6 blastocysts. Utilizing standard morphology grading, all top quality embryos maintained an average euploidy rate of 49.4%, showing no statistical difference between day 5, 6 or 7. Meanwhile, poor quality day 5 blastocysts achieved a statistically higher ($p < 0.05$) euploidy rate at 44.5% compared to poor quality day 6 (28.9%) and day 7 (24.6%) blastocysts.
Limitations, reasons for caution: The option to continue development past day 6 holds promise to attaining more viable embryos. It is of concern that extending embryo culture may increase possible detrimental effects. Yet, it is unknown if these day 7 blastocysts are at higher risk for any genetic, developmental or congenital risks.
Wider implications of the findings: An additional day of embryo growth allows extended possibilities for *in vitro* development. The success obtained questions the universal ideology that human embryos must blastulate by day 6 to be viable, and further questions if implantation failures are a result of an asynchrony between embryo and uterus.
Trial registration number: None.

O-057 Time-lapse analysis of preimplantation embryos affected by single chromosome abnormalities in PGS cycles

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Study question: To analyze differences in morphokinetic development of preimplantation embryos affected by single chromosome aneuploidy in Preimplantation Genetic Screening (PGS) cycles.
Summary answer: A trend towards slower blastocyst formation time was found in embryos affected by monosomies involving the larger chromosomes (1–5) compared to smaller chromosomes (21–22).
What is known already: Standard morphology evaluation has been the most widespread approach in many laboratories worldwide to select the embryo with the higher implantation potential. Many aneuploid embryos were shown to be compatible with development until blastocyst stage and were proven to reach good morphology grades. After the introduction of time-lapse technology, is recently available a more deepen software analysis for the examination of embryo development which can take into account also the morphokinetic parameters. Several studies assessed the association between morphokinetics development and embryo aneuploidy, although a clear relationship is still unmarked.
Study design, size, duration: This retrospective study was performed from September 2012 to December 2015. The morphokinetic development of 2385 embryos obtained in 733 PGS cycles with trophectoderm biopsy and array Comparative Genomic Hybridization (aCGH), was analyzed. All embryos were individually cultured in a time-lapse incubator at 37°C, 6.0% CO_2, 5.0% O_2. Mean female age was 36.6 ± 4.07 years old. The morphokinetic data are expressed in hours ± standard deviation.
Participants/materials, setting, methods: Among single chromosome's aneuploidies, with both monosomies and trisomies represented almost equally, a comparison between two groups of affected embryos based on chromosome's size, was performed: embryos where the largest chromosomes (from 1 to 5) or the smallest chromosomes (21 and 22) are involved. The morphokinetic parameters observed were: IIPB extrusion, 2PN appearance, pronuclear fading, onset of 2- to 8-cell divisions, time between 3- and 4-cell stage, morulae (tM), blastulation (tB), expansion and hatching timing.
Main results and the role of chance: A total of 8816 oocytes were collected, 6412 MII were injected and 4719 fertilized (73.6%). On day-3, 4702 embryos were obtained (99.6%). On day 5–7, a total of 2546 blastocysts were obtained (54.1%); 2455 blastocysts were incubated with time-lapse technology. All of them were biopsied. The genetic result was conclusive for 2385 embryos (97.2%). A total of 70 embryos failed to amplify (2.8%); 927 embryos (38.9%) resulted euploid while 1458 embryos (61.1%) resulted aneuploid. Among these latter: 295 embryos (12.4%) were established monosomy; 224 embryos (9.4%) were trisomy diagnosed and 939 embryos (39.3%) had multiple chromosomal aneuplodies. Among monosomies, chromosomes from 1 to 5 or chromosomes 21 or 22 are involved in the same number of embryos (n = 50; 16.9%). Among trisomies, 30 embryos (13.4%) were identified concerning chromosomes from 1 to 5 and 45 embryos (20.1%) chromosomes 21 or 22. Only a trend toward slower development has been issued for morphokinetic analysis of embryos where monosomy involves larger chromosomes showing that the morulae and blastocyst stage are reached and fully expand slightly slower if compared to smaller chromosomes: tM_{1-5} = 97.0 ± 11.1, tM_{21-22} = 93.0 ± 9.1 (p = 0.057); tSB_{1-5} = 106.3 ± 10.8, tSB_{21-22} = 102.2 ± 10.2 (p = 0.062); tB_{1-5} = 114.5 ± 11.1, tB_{21-22} = 110.4 ± 9.9 (p = 0.056); tEB_{1-5} = 124.4 ± 12.5; tEB_{21-22} = 119.1 ± 11.7 (p = 0.058). No statistical differences were found for the remaining morphokinetic parameters.
Limitations, reasons for caution: Restrained number of embryos enrolled in this study represent a limit to the amplitude of such findings. Moreover there is still a large discordance in the literature regarding the usefulness of different morphokinetic parameters to predict blastocyst development and implantation potentials as well as the related ploidy status.
Wider implications of the findings: Morphokinetic parameters are increasingly applied as informative for embryo competence to implant. This biological methodology require newer efforts, as literature's never-ending evidences are controversial. This study is meant to put forward novel insights which may help to clarify those prognostic factors that can predict embryo viability and its ploidy status.
Trial registration number: Not available.

O-058 Evidence that differences between embryology laboratories can influence the rate of mitotic errors, leading to increased chromosomal mosaicism, with significant implications for IVF success rates

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Study question: Do individual IVF clinics have different rates of chromosome abnormality in the embryos they produce, and if so, what might explain the differences observed?
Summary answer: Aneuploidy rates vary significantly between clinics. This is largely attributable to differing mitotic error rates, leading to chromosomal mosaicism, likely associated with suboptimal embryo culture.
What is known already: It is widely assumed that the incidence of aneuploidy in human preimplantation embryos is patient specific, principally influenced by female age, and little affected by variations in IVF laboratory procedures. What little debate there has been has tended to focus on the impact of alternative stimulation procedures on the risk of oocytes experiencing a meiotic error, becoming chromosomally abnormal, and ultimately producing an aneuploid embryo after fertilisation. To date, no convincing evidence has been presented indicating any effect on aneuploidy rate of differing embryo culture procedures, equipment or consumables, yet this remains an intriguing possibility worthy of further investigation.
Study design, size, duration: A total of 623 blastocyst stage embryos, derived from seven different IVF clinics, were biopsied and subjected to comprehensive chromosome screening. These analyses were part of routine preimplantation genetic screening (PGS) undertaken at a single genetic reference laboratory.

Participants/materials, setting, methods: Trophectoderm (TE) biopsy was followed by whole genome amplification and cytogenetic assessment. In order to provide the most rigorous evaluation, a well-validated next-generation sequencing (NGS) methodology was utilised. NGS provides highly accurate detection of aneuploidy affecting any chromosome. Additionally, it allows losses and gains of chromosomal fragments (segmental aneuploidy) to be detected. Finally, NGS has the greatest dynamic range of any chromosome screening method currently available, thus allowing sensitive detection of mosaic chromosome abnormalities.
Main results and the role of chance: Analysis of large numbers of blastocyst stage embryos confirmed that overall aneuploidy rates, seen in individual clinics, were similar once variation in maternal age had been accounted for. This confirms that the incidence of meiotic error in oocytes and sperm is relatively unaffected by differences in the protocols employed. The use of NGS provided an opportunity to examine the incidence of chromosomal mosaicism, caused by errors that occur in the mitotic divisions following fertilisation. The evaluation of appreciable numbers of embryos for mosaicism using comprehensive chromosome screening methods has not previously been possible, due to technical limitations and the high cost of such analyses. Importantly, the proportion of TE samples with evidence of mosaicism differed significantly ($p = 0.009$) between the clinics involved in the study, varying from 32% to over 60%. In particular, two clinics consistently produced high levels of mosaic embryos. The fact that mitotic errors producing mosaicism occur during embryo culture, suggests that variation in laboratory procedures could be responsible for the observed differences. Laboratory optimisation in order to reduce mosaicism is highly desirable, since most clinics opt to discard such embryos. The loss of potentially viable embryos due to induced mosaicism will inevitably impact IVF success rates.
Limitations, reasons for caution: Conclusions were drawn from data obtained from seven fertility clinics. Although comparison of results revealed that two of the seven clinics had significantly higher rates of chromosomal mosaicism, it is unclear whether such variation is commonplace or represents a relatively rare occurrence, found in this set of clinics by chance.
Wider implications of the findings: This study provides the first indication that differences between embryology laboratories affect chromosome abnormality rates. This finding was only possible due to application of new cytogenetic methods. Most clinics discard mosaic embryos and consequently laboratories that produce more mosaics will have fewer embryos available for transfer, negatively affecting pregnancy rates.
Trial registration number: Not applicable.

O-059 Quantification of mitochondrial DNA in preimplantation embryos: a tool to predict implantation potential of chromosomally normal embryos

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Study question: Can measurement of the mitochondrial DNA (mtDNA) content in preimplantation embryos improve the identification of the most likely euploid embryo resulting in a baby?
Summary answer: Low levels of MtDNA are related with increased potential of an embryo to successfully implant and result in a baby.
What is known already: MtDNA is a high copy-number, maternally inherited genome that codes for a small number of essential proteins involved in oxidative phosphorylation, metabolism and apoptosis. MtDNA molecules are very heterogeneous and allow cells to process energy very differently and the difference is able to differently regulate cell fate. Evidence form several mammalian species indicates that mtDNA content may cause a high level of developmental retardation and arrest of preimplantation embryos. However, there is limited data related to mtDNA content and its clinical relevance in the human embryo obtained during preimplantation genetic screening (PGS)/IVF.
Study design, size, duration: This study investigated the mtDNA, and nuclear DNA content in 96 blastocysts obtained from previously performed PGS cycles. These included 23 euploid embryos, 63 aneuploid, 10 mosaic and 5 degenerated embryos. The mtDNA copy number was correlated with blastocyst grading and clinical outcome (implantation, miscarriage, and live birth rate) after euploid embryos transfer.
Participants/materials, setting, methods: All embryos were cultured to blastocyst stage. After trophectoderm biopsy, and Whole Genome Amplification (WGA), mitochondrial and chromosomal DNA copy number variation were examined simultaneously with next generation sequencing (NGS). Analysis was accomplished with BlueFuse Multi software. mtDNA copy numbers was based on the observed ratios of sequence coverages between mtDNA and autosomal DNA.
Main results and the role of chance: The relative quantity of mtDNA was significantly lower in euploid embryos than those present in aneuploid embryos, and similar to those present in mosaic embryos. Among euploid blastocyst, the fully expanded (Grade 5 or 6) blastocysts had an mtDNA average value 1.6-fold lower than those with expansion grade 3. Additionally, mtDNA levels were elevated in embryos that degenerated, independent of chromosomal status ($P = 0.025$). Assessment of clinical outcomes after transfer of euploid embryos to the uterus revealed that blastocysts that successfully implanted and resulted in baby born, tended to contain the lower mtDNA quantities compared with those that failed to implant ($P = 0.007$) while those that resulted in biochemical pregnancy or early abortion showed an intermediate levels. mtDNA quantity threshold was established, above which implantation was never observed. Approximately 40% of the euploid blastocysts that did not implant or resulted in biochemical pregnancy had unusually high (above the threshold) mtDNA levels.
Limitations, reasons for caution: Due to the limited number of samples, further data and broad-based clinical application are required to better define the clinical relevance of mtDNA.
Wider implications of the findings: The results of this study suggest that increased mtDNA may be related to reduced viability and embryo degeneration. This data strongly suggests that mtDNA quantification has the potential to provide clinically relevant information additional to that provided by aneuploidy testing, representing a valuable biomarker of embryo viability.
Trial registration number: None.

O-060 Clinical implications of mitochondrial DNA quantification on pregnancy outcomes: a blinded prospective non-selection study

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Study question: Can quantification of mitochondrial DNA (mtDNA) in trophectoderm biopsy specimens provide information concerning embryo viability, potentially enhancing embryo selection and improving IVF treatment outcomes?
Summary answer: This study demonstrates that mtDNA levels are highly predictive of embryo potential. Euploid embryos of good morphology, but with high mtDNA levels fail to implant.
What is known already: Better methods of embryo selection are highly desirable in order to improve IVF treatment efficiency. Even the transfer of chromosomally normal embryos of high morphological grade cannot guarantee that a pregnancy will follow. Recently, the quantity of mtDNA in embryonic cells has been proposed as a new biomarker of viability – higher levels of mtDNA associated with reduced implantation potential. However, to date no prospective blinded studies have been undertaken to confirm this possibility. The current investigation involves the first evaluation of the predictive power of mtDNA quantification in a prospective, blinded, non-selection setting.
Study design, size, duration: mtDNA was quantified in 280 blastocysts, previously biopsied and shown to be chromosomally normal using preimplantation genetic screening (PGS). These were generated by 143 couples (average female age 37.2 years). All patients underwent IVF in a single clinic. The study took place in a blinded, non-selection manner – i.e., mtDNA quantity was not known at the time of single embryo transfer. The fate of the embryos transferred was subsequently compared to the mtDNA levels measured.
Participants/materials, setting, methods: Embryos were biopsied at the blastocyst stage. The trophectoderm samples obtained were subjected to whole genome amplification followed by comprehensive chromosome analysis using a next generation sequencing strategy (NGS). The same biopsy specimens were also tested using quantitative PCR, allowing highly accurate mtDNA quantification. After embryo transfer, the code used for blinding was broken and

analysis undertaken to reveal whether the amount of mtDNA had any association with blastocyst implantation.
Main results and the role of chance: mtDNA analysis of the 280 blastocysts revealed that 15 (5.4%) contained unusually high levels of mtDNA. At the time of writing, outcome data was available for 111 of the blastocysts, transferred after PGS results had been obtained but before mtDNA levels were known. All transfers involved a single chromosomally normal blastocyst of good morphology. Of these, 78 (70%) led to ongoing pregnancies, and all (100%) had mtDNA levels considered to be normal/low. The remaining 33 (30%) blastocysts failed to implant. Among these non-viable embryos there were 7 (21%) with unusually high levels of mtDNA. This meant that the ongoing pregnancy rate for morphologically good, euploid blastocysts, with normal/low levels of mtDNA was 76% (78/103). In contrast, the ongoing pregnancy rate for the same type of embryos, but with elevated mtDNA levels, was 0/7 (0%). This difference was highly statistically significant ($P < 0.0001$).
Limitations, reasons for caution: This study provides strong evidence that mtDNA quantification can serve as a valuable tool to improve the identification of viable blastocysts. However, to determine the true extent of any clinical benefits a randomised clinical trial will be necessary. Research is needed to improve understanding of the biology of mtDNA expansion.
Wider implications of the **findings:** This is the first study to evaluate the clinical impact of increased mtDNA in a prospective blinded manner. Results confirm that embryos with elevated mtDNA rarely implant, supporting its use as a viability biomarker. 76% of euploid embryos with normal/low mtDNA implanted vs. 70% for the cohort as a whole.
Trial registration number: Not applicable.

SELECTED ORAL COMMUNICATIONS

SESSION 17: BENCHMARKING ART

Monday 04 July 2016 **Hall 5 CB** **15:15–16:30**

O-061 How to measure success in ART: Using national registry data to inform patient, provider and policy decision making

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Study question: What are the most appropriate and relevant measures of ART success using population data?
Summary answer: Registry-based measurements of ART success vary widely and should reflect the perspective of the intended audience.
What is known already: There is no single measure of ART treatment success. "Dimensions" of success include efficacy, effectiveness, safety, and equity, while "perspectives" of success include that of the patient, providers, government and community. Traditional measures of success, such a live birth rate per cycle, are being challenged as new models of ART clinical practice emerge, particularly the shift to embryo banking and preferentially transferring frozen embryos. Furthermore, evolving models of care, increasing numbers of ART clinic providers worldwide and more informed patients, make the comparative reporting of ART success of increasing importance.
Study design, size, duration: A population-based study of ART treatments undertaken in 2009–2013 in Australia and New Zealand. During this period, 104,000 women undertook 340,000 fresh and frozen/thaw cycles resulting the 61,000 deliveries. A cohort of 39,698 women who had their first fresh ART cycle between 2009 and 2011 were followed through all fresh and thaw cycles to 2013 to calculate cumulative live birth rates based on a varying assumptions. Survival analysis was used to calculate age specific time to pregnancy.
Participants/materials, setting, methods: Information on ART cycles was sourced from the Australian and New Zealand Assisted Reproduction Database (ANZARD). ANZARD data are collected annually from all 80 fertility clinics in Australia and New Zealand as part of licencing agreements. ANZARD includes demographic characteristics of patients undergoing treatment, the types of ART procedures performed, and information on pregnancy and birth outcomes. ANZARD is a woman based data collection such that repeated cycles can be assigned to the same women.
Main results and the role of chance: The different dimensions (efficacy, effectiveness, safety, and equity) and perspectives (patient, health provider, funder, society) of measuring success in medicine are used as the foundation for demonstrating how ART success differs depending on how that data is portrayed. Detailed examples are demonstrated suing the ANZARD registry data.

Cycle-based success rates vary based on the selection of numerator and denominator, from 13.2% for Birth Emphasizing a Successful Singleton at Term (BESST) to 31% for clinical pregnancy per embryo transfer. The cumulative live birth rates per woman vary based on the definition of a cycle [a single cycle or a complete cycle (fresh plus frozen/thaw)], the age of the patient, and the assumptions made about women who discontinue treatment without achieving a pregnancy. The cumulative live birth rate varies by up to 30% points between conservative and optimistic assumptions about prognosis of women who discontinue treatment, and based on how a cycle is defined. Caution must therefore be taken when presenting these rates to patients.
Limitations, reasons for caution: This study used population-based data which is not reflective of an individual's ART success rates. Data was not available on length of infertility and BMI, and the definition of cause of infertility was not standardised. These factors are related to the likelihood of ART success and were not adjusted for.
Wider implications of the findings: The choice of measurement of ART success should reflect the intention of treatment and the target audience. If used appropriately, ART registry data is useful for informing patients about their likelihood of success over successive treatment cycles and time, for informing clinics about their performance, and for guiding public policy.
Trial registration number: NA.

O-062 The construction and the validation of an ART global score to quantitate an ART process

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Study question: Could we construct an objective ART Global Score to appreciate an ART process?
Summary answer: The construction and the validation of an ART Global Score were performed. This one was constructed with parameters presents in every database.
What is known already: In ART procedure it is very difficult to obtain an objective assessment of the procedure. The use of an ART database has become the norm, and the integration of a score calculated from the data in this database is then easy. However, the construction of an objective score is difficult. It must meet several requirements: be easy to calculate, built from a small number of variables, variables must be present in the database. Actually, none ART Global Score is available and has been validated.
Study design, size, duration: The ART Global Score was constructed using data from the MediFirst© database used in our center. The development of the score was conducted on a sample of 507 *In vitro* fertilization cycles carried out between January 2011 and July 2011. The validation of score was performed with 3801 cycles (from 2012 and 2013) obtained in our center (2741 cycles) and from another center (1060 cycles) in order to validate robustness of this one.
Participants/materials, setting, methods: Parameters were selected with univariate analysis (ROC curve, *t* test, …). Quantitative data were transformed to categorical variables (CHAID method classification). For each modality a weight was assigned. The weights were defined using logistic regressions and/or multiple linear regressions. The ART Global Score was the weight sum of each modality. The constraint was to obtain a linear relationship between ART Global Score and the pregnancy rate. The validation was performed with another set of ART cycles.

Main results and the role of chance: The ART Global Score is obtained from data from the woman, man and ART procedure. For Women, variables were age, BMI, initial LH and the rank of the puncture. For the man the selected variables are: BMI2222, sperm quality and sperm concentration after selection. And finally for the ART procedure: collected oocytes, Estradiol/oocyte, Gonadotropin/egg/day, 2 pronuclei oocytes on D1, fertilization rate, total embryos, embryos transferred, transfer stage and performing freezing. The ART Global Score could vary from 72 to 215. This ART Global Score was segmented into four classes depending on the pregnancy rates. There is a linear relationship between the ART Global Score and pregnancy rate ($r = 1.0$, $p < 0.001$). For an ART Global Score below than 90, the pregnancy rate was equal to 2.0%; for an ART Global Score between 91 and 115, the pregnancy rate was equal to 15.7%; for an ART Global Score between 116 and 155, the pregnancy rate was equal to 28.9%; and for an ART Global Score above 155, the pregnancy rate was equal to 43.7%. Similar results were obtained with the validating data; whatever the center and year, a linear relationship was obtained between ART Global Score and pregnancy rate.
Limitations, reasons for caution: This ART Global Score was constructed with an agonist ovarian stimulation, no antagonist stimulation was included. Some prognostic parameters were not included. AMH and sperm DNA fragmentation were not included as the measurement were not performed or not allowed for the majority of the cycles.
Wider implications of the findings: This ART Global Score could quantify objectively an ART process. Its scope is vast: clinical activity, research design, and quality indicator during accreditation process. The next step is the development of an algorithm to detect variables decreasing the ART Global Score, for a couple, in order to improve its management.
Trial registration number: None.

O-063 Performance heterogeneity of 41 IVF centers on cumulative live birth rate: measurement and practical implications for quality control and prognostic models

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Study question: Very few results are available concerning the comparison of Cumulative Live Birth Rate (CLBR) performance between IVF centers, and no validated methodology was proposed.
Summary answer: Our first investigation in France demonstrated a considerable relative variation of the mean CLBR rate of 52.3% and a maximum/minimum ratio of 2.34.
What is known already: Historical studies assessed the performance of centers essentially at cycle level. Strong between centers differences on CLBR were found, even after adjusting for patient characteristics (patient Mix). The ratio of maximum/minimum may exceed 3, and SD/mean coefficient of variation is near 50%. Explanatory causes are initial patient's selection, cancellation's rate, used ART and freezing techniques, number of embryos per transfer, and used statistics. As a higher number of cycles may compensate for lower LBR per cycle, center's performance was studied in terms of CLBR, but also on the total number of cycles required to achieve a live birth.
Study design, size, duration: We conducted a longitudinal observational national multicenter study on retrospective data in selecting couples starting IVF, and documenting all their successive fresh and frozen IVF cycles, during 3 years follow-up (Single Stage Cluster Sampling combined with within-center sequential recruitment). This study began in 2010 and was powered to provide a maximum error of 5% on CLBR, in planning a representative selection of 40 centers with a fixed recruitment of 100 couples per center.
Participants/materials, setting, methods: The performance Index (PI), ratio of LB on the total number of cycles, and CLBR constituted the two main measurements of performance. All the recruited patients were analysed on intent to treat basis. A mixed model was used in adjusting for women age, ovarian reserve and previous parity as fixed covariates, and center as random factor. The heterogeneity was tested as global (random intercept effect) and for subgroups (random effect of age, ovarian reserve).
Main results and the role of chance: Out of 58 selected IVF centers, 41 gave complete data and recruited 4081 couples, for a total of 8349 fresh and 2282 frozen IVF cycles. Our sample was characterized by a median age of 33 years (IQR = 7), mean BMI of 23.4 ± 4.7 and 18% smokers, 74.9% of childless couples, median duration of subfertility of 3 years (IQR = 3).

The unadjusted mean PI was 0.32 (range = 0.20; 0.49, IQ = 0.25, 0.37). By adjusting for Patient mix, we identified a decreasing quadratic effect of age, and a positive linear effect of previous LB parity and number of retrieved oocytes. By assuming the referent population as women aged 30 years, with 10 retrieved oocytes, the observed mean PI is 0.39 (0.37, 0.42). The standard deviation of the center effect SD = 0.05 [(0.03, 0.07), $p < 0.001$] provides a relative variation of 52.3%. The random effects of center on the number of oocytes and age were not significant.

The unadjusted mean CLBR was 0.48 (Range = 0.32; 0.64, IR = 0.41, 0.54), and some discordance was found among the centers between both values [R = 0.81, CI (0.76, 0.92)]. By adjusting for patient mix, the mean PI for the referent population is 0.59 corresponding to the above defined population of reference, with a relative variation of 39%.
Limitations, reasons for caution: Our model is sensitive to the center selection. In spite of random selection, centers refusing to participate may bias the results. Our first investigation is limited to France; results cannot be generalizable to other countries. In adjusting for patient Mix, some information was unavailable, in particular socio-cultural and environmental variables.
Wider implications of the findings: A strong heterogeneity of the performance among centers was found. Between-center collaboration may help: periodic data based on a mixed model constitutes a simple, accurate, and anonymous indication for each center. Moreover, adding center effect to patient mix provides a very accurate center-specific predictive model.
Trial registration number: It is not a clinical trial.

O-064 The impact of embryo transfer catheter retraction rate on fertility outcomes in intracytoplasmic sperm injection cycles: a randomized controlled trial

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Study question: Does retraction of embryo transfer (ET) catheter following a 30-s waiting period at the end of ET process affect pregnancy and clinical pregnancy rates in ICSI/ET cycles?
Summary answer: The retraction of ET-catheter following a 30-s waiting period inside the uterus has no positive effect on the rates in ICSI/ET cycles.
What is known already: Although many variables in ET such as catheter type, catheter loading technique, presence of blood on the catheter tip, have been studied as the determinants of a successful pregnancy, ET-catheter withdrawal rate has not been investigated.
Study design, size, duration: Prospective randomized controlled trial including 300 women aged <40 undergoing ICSI/ET cycles at our tertiary health care facility between October 2014 and March 2015. The patients were randomly enrolled to two groups as Group A and Group B. Following exclusion of 3 and 2 patients from the group A and B, respectively, the final statistical analyses included a total of 295 women.
Participants/materials, setting, methods: Group A included women in whom ET-catheter was retracted within the first 5-s; Group B consisted those in whom ET-catheter was retracted following a 30-s waiting-period at the end. As visualized by ultrasonography, women with and without fundal positioning of air bubble were evaluated as Group 1a and 1b, whereas those with a distance between catheter tip and fundal endometrial surface <15 and ≥15 mm were evaluated as Group 2a and 2b, respectively.
Main results and the role of chance: Demographic characteristics across all groups were comparable. Groups A and B did not significantly differ regarding pregnancy [32% ($n = 47$) and 32.4% ($n = 48$), respectively; $p = 0.933$] and clinical pregnancy [29.9% ($n = 44$) and 27.7% ($n = 41$), respectively; $p = 0.673$]

rates. In Group 1a (n = 207), pregnancy rate and clinical pregnancy rates were 33.8% (n = 70) and 28.4% (n = 25), respectively (p = 0.363), whereas in Group 1b (n = 88) were 30.4% (n = 63) and 25% (n = 22), respectively (p = 0.178). The distance between the catheter tip and fundal endometrial surface ≥15 mm (n = 144) was associated with significantly higher rates of pregnancy and clinical pregnancy than those with a distance <15 mm (n = 151) (26.5 versus 22.5%; p = 0.032 and 38.2 versus 35.4%; p = 0.014, respectively).

Limitations, reasons for caution: Lack of data regarding ongoing pregnancy, live birth and going home baby rates limit this study. Although air bubbles visualized at the time of ET are presumed to present the embryo position, it is only a surrogate of embryo itself.

Wider implications of the findings: In ICSI/ET, withdrawal of the ET catheter after a 30-s waiting inside the uterus or fundal migration of air bubble do not improve pregnancy or clinical pregnancy rates. Performing ETs with a distance of catheter tip to fundal endometrial surface ≥15 mm may be associated with better reproductive outcomes.

Trial registration number: N/A.

O-065 Is there a different effect of lifestyle intervention in subgroups of infertile obese women? Prespecified subgroup analyses of the LIFEstyle randomised controlled trial

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Study question: Is there a different effect of a 6-month lifestyle intervention preceding fertility treatment on reproductive outcomes in subgroups of obese infertile women?

Summary answer: Lifestyle intervention increased natural conception rate in anovulatory compared to ovulatory women, whereas rate of vaginal births of healthy singletons did not differ in subgroups.

What is known already: Obese women are at an increased risk of infertility and are less likely to conceive after fertility treatment. We previously demonstrated in the LIFEstyle study, that a 6-month lifestyle intervention preceding fertility treatment in obese infertile women did not increase the rate of vaginal births of healthy singletons at term within 24 months follow-up as compared to immediate fertility treatment (Relative Risk RR 0.77, 95% Confidence Interval CI 0.60–0.99). Natural conceptions of an ongoing pregnancy occurred more frequently in women who received a 6-month lifestyle intervention preceding fertility treatment (RR 1.6, 95% CI 1.2–2.2).

Study design, size, duration: Data of a multicentre, randomised controlled trial were used. Between 2009 and 2012, 577 obese infertile women were randomly assigned to a 6-month lifestyle intervention followed by fertility treatment (intervention group) or to immediate fertility treatment (control group). Subgroups were prespecified in the study-protocol based on frequently used cut-off values in the literature: age (≥36 or <36 years), ovulation status (anovulatory or ovulatory) and body-mass index (BMI, ≥35 or <35 kg/m^2).

Participants/materials, setting, methods: The rate of vaginal births of healthy singletons at term and the rate of natural conceptions of an ongoing pregnancy in the subgroups were calculated. Adjusted odds ratios (aOR) with corrections for baseline variables and the accompanying 95% CIs were provided. Logistic regression models with randomisation group, subgroup and the interaction between randomisation group and subgroup were used. A significant interaction was defined as a p-value <0.1. Follow-up period was 24 months after randomisation.

Main results and the role of chance: Data of 564/577 randomised women who had complete follow-up in the LIFEstyle study were analysed. The rates, % and aORs of a vaginal birth of a healthy singleton at term (Table 1) and natural conception of an ongoing pregnancy (Table 2) in women in the intervention group compared to women in the control group according to the subgroups and the accompanying p-values for interaction were:

Table 1. Rates and aOR of vaginal birth of healthy singletons at term.

	Rate (%)		$OR_{adjusted}$ (95% CI)	$p_{interaction}$
	Intervention	Control		
≥36 years	1/34 (2.9)	5/29 (17.2)	0.11 (0.00–6.23)	0.12
<36 years	75/246 (30.5)	95/255 (37.3)	0.90 (0.60–1.34)	
Anovulatory	37/123 (30.1)	52/140 (37.1)	0.82 (0.46–1.46)	0.91
Ovulatory	39/157 (24.8)	48/144 (33.3)	0.84 (0.49–1.44)	
BMI ≥ 35.0	43/175 (24.6)	61/180 (33.9)	0.78 (0.47–1.28)	0.62
BMI < 35.0	33/104 (31.7)	39/103 (37.9)	0.92 (0.49–1.76)	

Table 2. Rates and aOR of natural conceptions of an ongoing pregnancy.

	Rate (%)		$OR_{adjusted}$ (95% CI)	$p_{interaction}$
	Intervention	Control		
≥36 years	5/34 (14.7)	1/29 (3.5)	3.12 (0.27–35.9)	0.56
<36 years	68/246 (27.6)	45/255 (17.6)	2.17 (1.37–3.42)	
Anovulatory	35/123 (28.6)	16/140 (11.4)	4.15 (2.04–8.44)	0.02
Ovulatory	38/157 (24.2)	30/144 (20.8)	1.33 (0.73–2.41)	
BMI ≥ 35.0	47/175 (26.9)	33/180 (18.3)	1.93 (1.12–3.34)	0.37
BMI < 35.0	26/104 (25.0)	13/103 (12.6)	3.20 (1.42–7.22)	

Limitations, reasons for caution: This was a subgroup analysis of a randomised controlled trial and sample size determination of the trial was based on the primary outcome of the study. The study was not powered on analyses of subgroups or interaction tests. In general, interaction tests are prone to be underpowered.

Wider implications of the findings: The effect of a preconceptional lifestyle intervention is the strongest in anovulatory women and therefore it should be offered as first-line treatment for anovulation in obese women. An individual patient data analysis could give more insight on the effectiveness of lifestyle intervention in other subgroups of obese infertile women.

Trial registration number: The LIFEstyle study was registered at the Dutch trial registry (NTR 1530).

SELECTED ORAL COMMUNICATIONS

SESSION 18: COMPLICATIONS AND CONSEQUENCES

Monday 04 July 2016 **Hall 5 A** **15:15–16:30**

O-066 Late pregnancy complications and outcomes in women with threatened miscarriage: a systematic review and meta-analysis

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Study question: Are women with bleeding in early pregnancy at high risk of developing maternal and fetal complications that can contribute to term still-births?

Summary answer: Threatened miscarriage is associated with significantly increased risk of adverse maternal and fetal complications that can contribute to term still births.

What is known already: 16–25% of pregnancies suffer from early pregnancy bleeding and threatened miscarriage is diagnosed when along with the vaginal bleeding a viable pregnancy is noted on an ultrasound scan. Threatened miscarriage can be a reflection of placental development and dysfunction, which in turn explains the increased incidence of maternal and perinatal complications. A previously published systematic review and meta-analysis (Saraswat 2009) used a combination of both prospective and retrospective studies and has highlighted the need for more prospective studies in this field. Ever since more prospective studies have been published and therefore warrants an updated systematic review.
Study design, size, duration: This is a systematic review and meta-analysis of late pregnancy complications and outcomes in women with threatened miscarriage at less than 24 weeks of gestation (1946–2015). After thorough literature search and review, 11 prospective studies were found to be eligible for quantitative meta-analysis including 32,063 women.
Participants/materials, setting, methods: The electronic database search included Medline (1946 to December 2015), Embase (1980 to December 2015), Cochrane library, ClinicalTrials.gov and bibliographies of retrieved primary articles. Key MESH and Boolean terms were used for the search. Data extraction and collection was performed by two authors independently. Quality assessment of the individual studies was done using the Newcastle–Ottawa Scale and statistical analysis performed using the Cochrane systematic review manager 5.3 and Stata vs. 13.0.
Main results and the role of chance: The meta-analysis has shown that women with threatened miscarriage are at significantly increased risk of having preterm birth (OR 3.83; 95% CI 3.37–4.35); placental abruption (OR 4.29; 95% CI 2.72–6.75); placenta praevia (OR 4.9; 95% CI 2.34–10.29) and pregnancy induced hypertension (OR 5.79; 95% CI 4.56–7.34). They are also at an increased risk of still birth/intra uterine death (OR 1.97; 95% CI 1.21–3.19), pre-eclampsia (OR 2.6; 95% CI 2.01–3.36) and intra uterine growth retardation (OR 1.64; 95% CI 1.31–2.01). Threatened miscarriage also contributes to other complications like preterm pre-labour rupture of membranes (OR 2.62; 95% CI 2.1–3.28), low birth weight (OR 1.58; 95% CI 1.31–1.89), increased rate of caesarean section (OR 2.85; 95% CI 2.56–3.16), neonatal asphyxia (OR 1.78; 95% CI 1.1–2.89) and congenital anomalies/fetal malformations (OR 1.59; 95% CI 1.19–2.12).
Limitations, reasons for caution: The quality of some of the included studies is a limitation of this meta-analysis. Also potential confounding variables like maternal age, ethnicity, previous obstetric history, BMI are not accounted for, which could contribute to bias.
Wider implications of the findings: Threatened miscarriage can be associated with significant maternal and perinatal complications, therefore these women should be offered increased surveillance in the antenatal period. In light of the recent MMBRACE: UK perinatal mortality surveillance report 2015, there is a strong need to recognize and monitor factors contributing to still birth.
Trial registration number: Not applicable.

O-067 Risk factors for ectopic pregnancy in assisted conceptions: secondary analysis of human fertilization and embryology authority (HFEA) data

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Study question: What are the risk factors for ectopic pregnancy (EP) in a population receiving fertility treatment?
Summary answer: Tubal disease, low sperm motility, and increasing number of embryos transferred were associated with increased risk of EP.
What is known already: The prevalence of ectopic pregnancies has increased in recent times. It is thought that assisted reproductive technology may have contributed to this risk increase. Yet the reported risk of EP among the ART population varies between 0.8 and 8.6 versus 1% in the general population. This reported increase could be due to earlier diagnosis of pregnancy in the ART population.
Study design, size, duration: Case control study design was used to analyse HFEA data for first cycles only between 1998 and 2012. There were 99,528 pregnancies analysed of which 1,285 were ectopic. The risk factors assessed included age of women, year of procedure, primary or secondary infertility, duration of infertility, causes of infertility, type of ART procedure, fresh or frozen cycle and number of embryos transferred.
Participants/materials, setting, methods: All first cycles of IVF or DI conducted in the United Kingdom between 1998 and 2012 collected in the anonymised HFEA database were analysed. Univariate and multivariate logistic regression was conducted to calculate unadjusted and adjusted odds ratios with 95% confidence intervals for EP associated with each of the risk factors.
Main results and the role of chance: The incidence of ectopic pregnancy in this population was 1.31% (95% Confidence Intervals 1.30, 1.32). Tubal disease was strongly associated with the risk of EP in both univariate [1.63 (95% CI 1.37, 1.93)] and multivariate [1.89 (95% CI1.59, 2.25)] analyses. Low sperm motility [adjusted OR 1.90 (1.23–2.94)], was also associated with risk of EP. Risk of EP increased linearly with increasing number of embryos transferred – adjusted OR 1.18 (95% CI 1.02–1.38), 1.35 (1.21–1.50) and 1.02 (0.85–1.22) for two, three and four or more embryos transferred respectively.

On univariate analysis, increasing age of women was associated with reduced risk of EP, but this association was no longer significant on multivariate analysis. The odds of EP were not significantly increased with increasing year of procedure or duration of infertility. Secondary versus primary infertility was also not found to be a significant risk factor for EP in the adjusted model.

Analysis of a large population based database such as the HFEA database is likely to yield statistically significant results that may or may not be clinically significant. However, the strong associations seen with some of the risk factors in this analysis are also biologically plausible and thus unlikely due to chance.
Limitations, reasons for caution: Due to the anonymization of the HFEA database, multiple cycles belonging to the same woman cannot be identified. We accounted for this by analysing only the first cycles. There was also no information about any previous history of EP, the single most important risk factor for EP.
Wider implications of the findings: EP is a leading cause of maternal mortality and morbidity. Its association with ART procedures can further reduce chances of treatment success. Single embryo transfers may reduce the risk of ART associated EP.
Trial registration number: Not a trial.

O-068 Prevention of adhesions post (spontaneous) abortion (PAPA study); a randomized controlled trial evaluating application of hyaluronic acid (HA)

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Study question: Does intrauterine application of Hyalobarrier® Gel Endo (HA) in women undergoing a recurrent D&C, reduces the amount of intrauterine adhesions (IUAs).
Summary answer**:** Intrauterine application of HA following conventional D&C for miscarriage, in women with at least one previous D&C, significantly reduces the cumulative rate of IUAs.
What is known already: Approximately 15–20% of all clinically recognized pregnancies will end in a miscarriage; a pregnancy that fails to progress before 20–24 weeks of gestation. IUAs, defined as fibrous strings at opposing walls of the uterus or cervix may result in menstrual disorders and infertility while pregnancy is frequently complicated; because of the possible implications, prevention is important.
Study design, size, duration: From December 2012 through April 2015, we performed a multicenter, open-label, randomized controlled trail at one university and seven university-affiliated teaching hospitals. Women, undergoing a recurrent D&C were randomized preoperatively by a web based computer program and allocated to receive either Hyalobarrier ® Gel Endo (prevention group) or nothing (control group) post operatively. A hysteroscopic procedure was scheduled 8–12 weeks after the D&C-procedure to evaluate the uterine cavity.
Participants/materials, setting, methods: Women with at least one previous D&C in history, a group with an increased risk of clinically significant adhesion

formation, were eligible to participate if they were diagnosed with a miscarriage and planned for a surgical intervention (D&C). Women suspected of a molar pregnancy and with severe signs of infection were excluded.
Main results and the role of chance: 152 women were randomized; 78 women were assigned to the prevention group and 74 to the control group. The vast majority of women (98.7%) assigned to the prevention group, received the study intervention. The baseline characteristics among participants were similar. IUAs were observed in 10 of 77 women (13.0%) in the prevention group compared to 22 of 72 women (30.6%) in the control group (RR 0.43; 95% CI: 0.22–0.83, $p = 0.01$). The number needed to treat (NNT) to benefit is 6. Mean adhesion scores, based on the extent of uterine cavity involvement, type adhesion and menstrual pattern was significantly lower in the prevention group compared to control; respectively 2.80 versus 4.41 (MD − 1.61, 95% CI − 3.16 to − 0.06, $p = 0.04$). No complications or adverse events were reported related to the application of Hyalobarrier® Gel Endo.
Limitations, reasons for caution: We were not able to blind the surgeon performing the D&C procedure, we made sure that the hysteroscopic examiner was unaware of the assignment: the examiner may not have participated or contributed to the initial D&C procedure of the woman being examined.
Wider implications of the findings: There is an association between the presence and extent of IUAs and long-term reproductive complications but few studies addressing the link are available; prevention is important. The effect of prevention on reproductive outcome will emerge from ongoing work.
Trial registration number: The Netherlands Trial register (NTR 3120).

O-069 Effectiveness of treatment in the secondary prevention of obstetric complications in the antiphospholipid syndrome: systematic review and meta-analysis

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Study question: Which treatments are the most effective for the secondary prevention of obstetric complications in pregnant women with history of obstetric antiphospholipid syndrome (APS)?
Summary answer: This meta-analysis shows a trend towards superiority of aspirin + low-molecular-weight heparin vs. aspirin + unfractionated heparin in preventing miscarriages, and no effect of antithrombotic treatments on placenta-mediated complications.
What is known already: Several recent meta-analyses (Ziakas et al., 2010; Mak et al., 2010) were performed to assess whether antithrombotic treatments reduce the risk of miscarriages and placenta-mediated complications in the secondary prevention of obstetric APS pregnant women. However, even though the current practice is the use of low-molecular-weight heparin (LMWH), data on substitution of unfractionated heparin to LMWH remain inconclusive. Furthermore, the impact of antithrombotic therapy on the risk of placenta-mediated complications is still controversial.
Study design, size, duration: We selected studies that included pregnant women with obstetric APS (at least two miscarriages and the presence of antiphospholipid antibodies), and describing exposure to different treatments [heparin including unfractionated heparin and LMWH, aspirin, IV immunoglobulin (IVIG)]. The primary endpoint was the incidence of early miscarriages (before 14 weeks of gestation). Secondary outcomes were obstetric complications related to antiphospholipid syndrome: late miscarriages, preeclampsia, intra uterine growth retardation (IUGR), and premature delivery.
Participants/materials, setting, methods: To ensure the quality of the methodology, the MOOSE/PRISMA criteria have been met at all stages of the development of this meta-analysis. We searched the Cochrane Library, EMBASE, MEDLINE and reference lists of eligible studies from inception to September 2015, without any restriction. We also interviewed the ClinicalTrials.gov database for unpublished articles and meeting abstracts. Two reviewers independently extracted study characteristics and outcome data. Estimates were pooled using random effects models and sensitivity analyses.
Main results and the role of chance: Of 2018 identified abstracts, 26 primary studies (7 cohorts, 1 case-control, 18 randomized control trials) met inclusion criteria, including 1269 APS patients. Compared to aspirin alone, heparin with aspirin allows a 50% reduction of early miscarriages in APS [4 studies, 695 patients, OR = 0.46 (CI 95%; 0.31–0.69)]. Furthermore, our meta-analysis shows a very strong trend to superiority of LMWH compared with unfractionated heparin [3 studies, 138 patients, OR = 0.46 (0.21–1.01)] in the prevention of miscarriages, however without reaching statistical significance. The results on the effectiveness of conventional antithrombotic treatments in the prevention of placenta-mediated complications (late miscarriages, preeclampsia, and IUGR) did not reach statistical significance. However, adding IVIG to LMWH and aspirin was associated with an 85% decreased risk of pre-eclampsia [2 studies, 139 patients, OR = 0.15 (0.03–0.88)]. Finally, among all treatments, the association of aspirin and LMWH in comparison with aspirin alone was associated with an 80% decreased risk of prematurity [2 studies, 173 patients, OR = 0.21 (0.07–0.61)].
Limitations, reasons for caution: Some primary studies are non-randomized cohort studies. This could have impacted the internal validity of some results. Furthermore this meta-analysis was performed on published data.
Wider implications of the findings: Further randomized trials or an individual patient-level meta-analysis are needed to confirm these results and identify which treatments are effective in the secondary prevention obstetric APS manifestations especially those placenta-mediated.
Trial registration number: None.

O-070 Recurrent pregnancy loss is not associated with an increased breast cancer risk

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Study question: Are women with recurrent pregnancy loss (RPL) (two or more miscarriages) at a higher risk to develop breast cancer over time?
Summary answer: Women with RPL are neither at a higher risk nor at a lower risk to develop breast cancer over time.
What is known already: Some studies suggest that RPL might be due to increased instead of decreased levels of angiogenic factors with consequently superfertility but because of allowance of implantation of also poor quality embryos the result is more clinical miscarriages. As breast cancer progression is also associated with increased angiogenesis, we wondered if RPL might be associated with an increased risk of breast cancer.
Study design, size, duration: The nationwide Dutch OMEGA cohort comprised 13,715 women who received *in vitro* fertilization between 1983 and 1995 and completed a questionnaire between 1997 and 2000. In total, 831 women refused linkage with disease registries and 4,318 women were excluded (diethylstilbestrol, nulliparous without miscarriages) leaving 8,340 women in our analysis. Breast cancer incidence was updated until January 2014 through linkage with the Netherlands Cancer Registry. Follow-up started at questionnaire completion. Mean follow-up time was 15.2 years.
Participants/materials, setting, methods: Women were classified according to their number of miscarriages as follows; zero miscarriages (5,871; 70.4%), one miscarriage (1,799; 21.6%) and RPL (670; 8.0%). In total, 298 breast cancer cases were identified. Breast cancer risks were estimated using a Cox regression model. Sensitivity analyses were done excluding all nulliparous women with miscarriages and within miscarriage groups.
Main results and the role of chance: Within the group without miscarriages 219 women had breast cancer, 55 women with one miscarriage, and 24 women with RPL had breast cancer. Hazard ratios (HR) were calculated for one miscarriage [0.92, 95% confidence interval (CI) 0.68–1.27] and RPL (1.06, 95% CI 0.68–0.164). When restricting to only parous women HR for one miscarriage and RPL remained similar. Within the one miscarriage (1.38, 95% CI 0.76–2.50) and RPL (2.72, 95% CI 0.93–7.99) group parous women had higher HRs for breast cancer risk, although not significant. When comparing all different groups (nulliparous women with one miscarriage, nulliparous women with RPL, parous women with zero miscarriages and parous women with RPL) with each other taking parous women with one miscarriage as reference group there were no significant findings. HRs for nulliparous women with one miscarriage

were 0.64 (95% CI 0.38–1.07), for nulliparous with RPL 0.43 (95% CI 0.16–1.15) and for parous women without miscarriages 0.88 (95% CI 0.63–1.23).
Limitations, reasons for caution: Information about miscarriages was obtained from the questionnaires and therefore may have been prone to recall bias. However, this is not very likely, because we expect recalling the miscarriages not to be dependent on having breast cancer in the future.
Wider implications of the findings: Since our results refute any relation between occurrence of breast cancer and previous RPL we cannot assume simple links between angiogenic features of breast cancer development and that of RPL and possible superfertility. We found a non significant lower breast cancer risk in nulliparous women with miscarriages.
Trial registration number: Not applicable.

SELECTED ORAL COMMUNICATIONS

SESSION 19: LOSS AND DISTRESS IN INFERTILITY. TREATMENT AND BEYOND

Monday 04 July 2016 **Hall 3 AB** **15:15–16:30**

O-071 He said, she said: gender differences in causal explanations for infertility/fertility problems

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Study question: Do men and women have different causal explanations for their fertility problems?
Summary answer**:** Men and women blame chance and medical problems for infertility. Men also blame their lifestyle (sexual practices) and women their reproductive choices (birth control, abortion).
What is known already: It is commonly believed that women take the blame for a couple's fertility problems in order to protect their partner's self-esteem or sense of masculinity. But research tells a different story. Some studies show women are more likely than men to blame their fertility problems on their own past behaviours. Other studies show the opposite – with men more likely to blame their fertility problems on their own past behaviours and women to blame destiny or fate.
Study design, size, duration: Mixed method design. A cross-sectional sample completed the causal attribution questions of the International Fertility Decision-Making Study (IFDMS, translated into 12 languages) over a 9-month period online, through a social research panel or in fertility clinics. Inclusion criteria were that respondents were between age 18 and 50, partnered, and trying to conceive for 6+ months.
Participants/materials, setting, methods: 10,045 respondents (1,690 men, 8355 women) from 79 countries participated. Respondents were 31.8 years and trying to conceive for 2.8 years. Participants rated on 5-point Likert-scales to what extent they perceived their fertility problems to be due to something they/their partner did or to other factors and to describe their reasons for making this attribution. 29.7% (337 men, 2651 women) provided 3,900 text responses to these questions. Textual data was analysed using thematic analysis.
Main results and the role of chance: In quantitative ratings, men and women most commonly attributed their fertility problems to chance or bad luck and medical conditions. Men were significantly more likely than women to attribute their fertility problems to something their partner had done, their or their partner's lifestyle, or their partner's age. Women were significantly more likely to cite chance or bad luck, medical or emotional conditions, God's will, or their age as causes of their fertility problems. Thematic analysis of qualitative results found that women tended to blame their fertility problems on their reproductive choices (most commonly abortion or birth control use), to failure to change lifestyle behaviors (e.g., weight, smoking), and to their partner's lack of readiness for parenthood (e.g., ambivalence about starting a family, lack of desire to be a parent). Women expressed regret and helplessness about these causes, which they perceived led to delayed childbearing and subsequent fertility difficulties. In contrast, men believed their lifestyle behaviours (e.g., smoking, specific sexual practices) and their partner's previous abortions were the cause of their fertility problems, and did not express feelings of regret or helplessness.
Limitations, reasons for caution: Respondents were self-selected and results principally reflect views of highly educated women with prior exposure to fertility health services.
Wider implications of the findings: Education campaigns should address misconceptions about the impact of abortion and birth control on future fertility. Future research should address that men's readiness for parenthood significantly influences the decision to start a family. Lifestyle behaviour change interventions should target feelings of ambivalence along with ways to reduce future regret.
Trial registration number: N/A.

O-072 Endometriosis is associated with depression and anxiety still at premenopausal age – a population based cohort analysis

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[2]*University of Oulu, Center for Life Course Health Research, Oulu, Finland*
[3]*Helsinki University Hospital, Department of Obstetrics and Gynaecology, Helsinki, Finland*

Study question: Is endometriosis associated with long lasting psychological consequences with increased mental distress (depression and anxiety) in early adulthood and in premenopause?
Summary answer: Endometriosis associates with higher rate of depression/anxiety symptoms and diagnosed depression at ages 31 and 46 compared with controls.
What is known already: Women with endometriosis have been shown to present with an increased prevalence of depression and anxiety. Infertility and chronic pain symptoms associated with endometriosis are considered the most important explanatory factors and the symptoms may prevail for years. However, there are only few longitudinal studies on age-related mental distress in these women and population based analysis are lacking.
Study design, size, duration: The study population was a prospective follow-up of a large national birth cohort including 12,231 subjects (5889 women). A postal questionnaire screening previously diagnosed endometriosis was collected at age 46. The symptoms for mental distress and depression diagnosis were screened both ages 31 (n = 4523) and 46 (n = 3706). The questionnaire identified 258 (7.7%) women with endometriosis and 3090 controls. We also analyzed the use of medication for depression and anxiety.
Participants/materials, setting, methods: Both 31 and 46-year questionnaire included Hopkins Symptom Check-list 25 (HSCL-25), a screening tool for depression/anxiety symptoms. The women were also asked whether they had been diagnosed or treated for depression. Furthermore, the study group was also screened for their current medication. The associations were calculated using Pearson Chi-square and Mann–Whitney U test when appropriate. The results were adjusted for BMI, smoking, hormonal contraceptive use, infertility and socioeconomic status (SES) with binary logistic regression model.
Main results and the role of chance: HSCL-25 revealed that women with endometriosis had more symptoms for mental distress both at age 31 [median (interquartile range): 1.32 (0.36) vs. 1.28 (0.32), p = 0.032, respectively] and 46 [1.32 (0.48), 1.26 (0.36), p = 0.018]. The HSCL-25 anxiety score was higher in women with endometriosis compared with controls up till perimenopause [31 years, 1.30 (0.4) vs. 1.22 (0.3), p = 0.013; 46 years, 1.30 (0.4) vs. 1.20 (0.3), p = 0.006]. Higher proportion of women with endometriosis had anxiety score in the upper quartile (31 years, 43.2 vs. 33.9% p = 0.003; 46 years, 41.2 vs. 31.8% p = 0.002) compared with controls. A similar trend was observed in the HSCL-25 depression score [31 years, 1.33 (0.4) vs. 1.27 (0.46), p = 0.065; 46 years, 1.33 (0.53) vs. 1.27 (0.46), p = 0.05].

History of endometriosis was associated with higher lifetime incidence of depression diagnosis compared with controls (31 years, 9.7 vs. 4.1%, $p < 0.001$; 46 years, 21.6 vs. 13.1%, $p < 0.001$). Furthermore, the prevalence of women having depression diagnosis both at age 31 and 46 was higher among women with endometriosis (6.3 vs. 2.4%, p = 0.001).

However, there was no difference between the study groups regarding the current use of anxiety (31 years, 2.3 vs. 1.9%, p = 0.816; 46 years, 12.4

vs. 10.7%, $p = 0.403$) or antidepressant medication (31 years, 1.6 vs. 1.2%, $p = 0.767$; 46 years 10.5 vs. 9.4%, $p = 0.580$).

The adjustments for smoking, SES, BMI, hormonal contraceptive usage and history infertility did not affect the results.

Limitations, reasons for caution: The diagnoses of endometriosis were self-reported. However, 37.7% of cases were available for validation using hospital patient records. The hospital register data confirmed endometriosis diagnosis for 76.3% of self-reported cases and of them 90.1% were diagnosed with laparoscopy.

Wider implications of the findings: This unique population based data demonstrates increased mental distress in women with endometriosis until premenopausal age implying the disease having long lasting consequence on women's mental well being. In clinical practice the women should be screened for mental distress beyond fertile age and offered with appropriate care.

Trial registration number: –.

O-073 Psychological morbidity of women who experience miscarriage: impact of a specific counselling training for residents using simulation

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Study question: Does a specific training for residents in Gynecology and Obstetrics using simulation in the announcement of a miscarriage may reduce women's distress and psychological morbidity?

Summary answer: Training using simulation for residents in the announcement of a miscarriage improves the wellbeing and diminishes the psychological morbidity of patients after a pregnancy loss.

What is known already: Structured simulations are used for critical part of health professions education at every level. Breaking the bad new of a miscarriage is a challenging situation for doctor. Indeed, an early miscarriage is usually associated with a psychological distress. Women can express different feelings such as grief, guilt, post-traumatic stress, anxiety or depression. The announcement of a pregnancy loss and the information given during the consultation may have a major role on the psychological outcomes of the patients. The majority of patients underscore the need for a more considerate care of the healthcare team in the management of early miscarriage.

Study design, size, duration: A "before and after" prospective study was conducted in a French teaching hospital. All women who experienced an announcement of an early pregnancy loss in our gynecological department emergencies from May 2014 to May 2015 were included. At all, 72 patients were included, 45 before and 27 after the training.

Participants/materials, setting, methods: At half-time of the training course, all the six residents who were in charge of emergency cases over the study period attended the training which consisted in an "*in situ*" simulation of early miscarriage announcement. An auto-questionnaire was sent to all patients 8 weeks after the emergency consultation. This questionnaire included specific questions in order to assess the feelings at the end of the consultation and to assess the perinatal bereavement using a validated scale.

Main results and the role of chance: The mean age of the patients was 31 years. The mean term at miscarriage was 8 weeks gestation. The feeling of the patients experiencing a miscarriage was improved after a specific counselling training using simulation. Thus, the lack of availability and the indifference of doctors regarding the pregnancy loss are significantly less experienced by patients after training ($p = 0.03$ and $p = 0.04$ respectively). The information given during the consultation also appear to be more complete, with significantly fewer patients consulting another doctor after the consultation for answering their questions ($p = 0.04$). Concerning the perinatal grief scale, women before training experienced more intense grief scores after a miscarriage than in the post-training group (57.3 vs. 39, $p = 0.02$).

Limitations, reasons for caution: Our study, despite its prospective design, may suffer of a lack of power due to the small sample size. Another limit is the generalizability of the results due to the monocentric design of the study. Further studies are needed to confirm our data's.

Wider implications of the findings: A training for the residents in the announcement of a miscarriage seems to be associated with a better psychological morbidity on women. All gynecologists (seniors and juniors) should be able to receive training in the announcement of bad news in order to reduce the psychological implications of a miscarriage.

Trial registration number: None.

O-074 A meta-analysis of maternal psychosocial effects of twins and multiple births following assisted conception or natural conception/ART singleton births

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Study question: Is maternal psychological health adversely affected by twins and multiple births, by assisted conception or both?

Summary answer: Mothers of ART multiples are significantly more likely to experience depression and stress compared to mothers of ART singletons, but not naturally conceived (NC) multiples.

What is known already: Research on adverse maternal psychosocial consequences of twins and multiple births following assisted reproductive technology (ART) is conflicting and no definitive evidence for the effects of ART and of multiple births on maternal psychological health exist.

Study design, size, duration: Meta-analytic data were analysed using random effects models.

Participants/materials, setting, methods: A bibliographic search was undertaken using PubMed, PsycINFO, CINAHL, ScienceDirect, and hand searches of reference lists up to September 2014. Where insufficient data was available, authors were contacted, which resulted in the inclusion of further unpublished data using PRISMA The review of abstracts and papers was informed following PRISMA and MOOSE guidelines. The quality criteria checklist included the recommendations of Cochrane Collaboration.

Main results and the role of chance: Eight papers (data from 2993 mothers) were included in the meta-analysis. Mothers of ART multiples were significantly more likely to experience depression (standardised mean difference $d = 0.198$, 95% CI 0.050–0.0345, $z = 2.623$, $p = 0.009$; heterogeneity $I^2 = 36.47\%$, $p = 0.146$), and were significantly more likely to experience stress (standardised mean difference $d = 0.177$, 95% CI 0.049– 0.305, $p = 0.007$; heterogeneity $I^2 \leq 0.01\%$, $p = 0.535$) than mothers of ART singletons. Meta-analysis of psychological distress (combined stress and depression) in mothers of ART multiples were no different from mothers of NC multiples (standardised mean difference $d = 0.371$, 95% CI 0.153–0.895 $p = 0.165$; $I^2 = 86.962\%$, $p = 0.001$). Similarly, when depression was analysed separately, no differences between mothers of ART multiples and mothers of NC multiples were found ($d = 0.152$, 95% CI 0.179–0.483: $z = 0.901$: $p = 0.368$; $I^2 = 36.918\%$, $p = 0.208$).

Limitations, reasons for **caution:** This systematic review and meta-analysis has confirmed that mothers of ART multiple births are significantly more likely to experience depression and stress compared to mothers of ART singletons, but the impact of the role of ART and multiple births is not yet resolved.

Wider implications of the findings: Clinicians should be aware of the likelihood of stress and depression in mothers of multiples and in women undergoing ART, and use these data to deter multiple embryo transfers and encourage eSET as the effects were demonstrated well into the first few years post-partum.

Trial registration number: NA.

O-075 Psychosocial adjustment after failed fertility treatment: a meta-analytical and qualitative review

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Study question: How do patients adjust after failed fertility treatment?

Summary answer: Failed treatment results in worse mental-health and well-being. Adjustment depends on patients' ability to accept childlessness, make meaning of their situation and pursue new goals.

What is known already: One-third of fertility patients do not achieve parenthood with treatment. It is unclear if these patients adjust worse than those who do. Quantitative research shows mixed results but these can be explained by conceptual and methodological heterogeneity. Furthermore, quantitative studies have not identified the risk factors nor the psychosocial mechanisms at play during this period. Therefore there is no clear indication for the provision of psychosocial support after failed treatment and no knowledge about the therapeutic mechanisms it should target. However, several qualitative studies focused on patients' experiences during this period and these can inform about their support needs.

Study design, size, duration: Meta-analytical and systematic qualitative review of articles focusing on adjustment after failed treatment published since 1978 in 5 electronic databases.

Quantitative studies had to include group mean comparisons on mental-health (anxiety, depression, psychopathology, stress, etc.) or wellbeing (general-wellbeing, life satisfaction, quality-of-life, etc.) between patients who did failed fertility treatment and a control group (successful treatment, children after treatment).

Qualitative studies had to focus on psychosocial adjustment or experiences or feelings after failed fertility treatment.

Participants/materials, setting, methods: Screening, data extraction and critical appraisal procedures were done independently by the authors using predefined protocols.

Two meta-analyses were performed on mental-health and wellbeing with a random effect model. Primary outcome was Hedge's *g* (0.20, 0.50, 0.80 indicate small, medium, large effect-sizes).

A 3-stage thematic analysis of results reported in primary qualitative papers was implemented following guidelines for qualitative meta-synthesis (Thomas and Harden, 2008). First-order descriptive and second-order interpretative themes were extracted.

Main results and the role of chance: The search returned 5979 non-duplicate records, 119 were retained based on title and abstract and 9 quantitative (2085 patients from 8 countries) and 9 qualitative (267 patients from 6 countries) were included. Quality ratings indicate none low, seven moderate (78%) and two (22%) high-quality quantitative studies, and none low, eight moderate (73%) and three (27%) high-quality qualitative studies.

Six (67%) of the quantitative studies reported on mental-health and 7 (78%) on wellbeing. The meta-analysis showed that the failed group had worse mental-health [$g = -0.450$, $P = 0.002$, 95% CI (−0.734 to −0.267]; $I^2 = 85\%$, $P < 0.001$] and wellbeing [$g = -0.319$, $P < 0.001$, 95% CI (−0.439 to −0.198), $I^2 = 45\%$, $P = 0.001$] than the control group.

The qualitative review resulted in a total of 33 first-order themes that were grouped into 6 second-order themes: childlessness experience – individual, childlessness experience – relational and social, childlessness acceptance, pursuit of new life goals, meaning making, and fertility care perceptions and needs. These themes captured how patients' individual and relational experience and their care perceptions and needs changed over time and how this change was associated with how successful they were in engaging with three main psychological tasks: accepting childlessness, making meaning of their past and current situation and pursuing new fulfilling life goals.

Limitations, reasons for caution: This was a sound quant+qualitative meta-synthesis. However, research in this topic is emergent and few studies were included, mostly being descriptive and suffering from methodological limitations. Only 5 (28%) were of high-quality. The qualitative synthesis was based on published papers that included some level of interpretation of the data collected.

Wider implications of the findings: Results provide compelling evidence for the provision of psychosocial support after failed fertility treatment. Such support needs to target psychological mechanisms that are usually involved in coping with stressful life-events and loss, namely accepting the loss, making meaning of the past/present situation and building new life-goals for the future.

Trial registration number: NA.

SELECTED ORAL COMMUNICATIONS

SESSION 20: OVARIAN TISSUE CULTURE, CRYOPRESERVATION AND GRAFTING

Monday 04 July 2016 **Hall 3 DE** **15:15–16:30**

O-076 Improvement of ovarian tissue survival after cryopreservation with anti-apoptotic drugs supplementation of transport and freezing media

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[2]University of Liege, LBTD, Liege, Belgium

Study question: Does ovarian tissue supplementation with anti-apoptotic drugs during transport and cryopreservation improves ovarian tissue survival?

Summary answer: Addition of S1P or Z-VAD-FMK to transport and freezing media prior to ovarian tissue cryopreservation improves primordial follicular quality and the global tissue survival.

What is known already: Ovarian transplantation is avascular, resulting on tissue ischemia after grafting associated with reperfusion injury. This represents the main origin of follicular loss. In addition, freezing procedure induces tissue damage: fibrosis and alteration of the viability of both stromal and follicular cells within the ovarian samples. Apoptosis plays important role in cryo-injuries, mainly by the activation of caspases and Fas systems. Indeed, caspase activation was observed in frozen-thawed tissue with preserved architecture.

Study design, size, duration: Sheep ovaries were transported, prepared and frozen in solutions containing vehicle or anti-apoptotic drugs [Z-VAD-FMK or sphingosine-1-phosphate (S1P)]. After thawing, the ovarian cortex was cultured during 2 or 6 days. Six ovarian fragments were analyzed in the each 4 groups: Z-VAD-FMK, Z-VAD-FMK control, S1P and S1P control. These 4 groups were investigated at 4 timings: fresh, frozen-thawed, 2 days and 6 days culture.

Participants/materials, setting, methods: Ovaries of 4 ewes, 4 and 5 months old, were collected after euthanasia. The anti-apoptotic drugs included 10 µM Z-VAD-FMK diluted in DMSO and 10 µM S1P diluted in 0.3M NaOH. In the control groups, the anti-apoptotic drugs were replaced with the appropriate vehicles DMSO or NaOH. Follicular density, morphology and tissue proliferation analyses were performed on paraffin histological sections.

Main results and the role of chance: Only primordial follicles were analyzed due to a very low number of primary and secondary follicles. Transitional follicles were considered as primordial.

To limit the effect of the heterogeneous distribution of the follicular pool within the ovarian cortex, 12 sections per ovarian piece, which covered the entire fragment, were analyzed as previously described by our team. After the outcomes were logarithmically transformed, a linear mixed model was fit to the data to test differences between the treatments and the timings. In this model, the ovarian fragment was introduced as a random factor. To correct for multiple comparisons and to avoid type I errors, the level of statistical significance was set at $p = 0.01$.

After 2 days of culture, S1P improved the quality of primordial follicles; higher densities of morphologically normal ($p = 0.0005$) and proliferative primordial follicles were found compared to control ($p = 0.0087$). Z-VAD-FMK displayed similar effects by preserving global primordial follicular density, but after 6 days of culture ($p < 0.0001$ for global density, $p = 0.0035$ for morphologically normal follicles and $p = 0.0055$ for proliferative follicles). Z-VAD-FMK also improved global cell proliferation after 2 and 6 days of culture, compared to control ($p < 0.0001$ for both).

Limitations, reasons for caution: This study was performed only on sheep ovarian tissue. The beneficial effects of Z-VAD-FMK and S1P in culture over the span of 2–6 days does not necessarily equate into evidence that this approach would allow better engraftment and/or maintenance of primordial follicle health after transplantation.

Wider implications of the findings: Our study demonstrates the beneficial effect of Z-VAD-FMK, and to a lesser extent S1P, on primordial follicles preservation after cryopreservation. These results must be confirmed in human ovarian cortex and after transplantation. Therefore, this model at present only provides a surrogate marker to encourage additional work in this area.

Trial registration number: None.

O-077 Attempts to improve human ovarian tissue grafting with novel matrices for tissue fusion and regeneration

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Study question: Can novel substances for tissue repair [small intestinal submucosa (SIS) and recombinant human collagen bioengineered in plant lines (Collage™)] improve outcomes of human ovarian grafting?
Summary answer: Collage promoted reduction in atretic follicles and in tissue apoptosis. It also increased follicular survival after grafting, while SIS supplementation did not benefit implantation.
What is known already: So far, 60 live births have been reported after grafting frozen–thawed human ovarian tissue to women. However, slow ovarian neovascularization after implantation leads to follicular loss. Therefore, methods to enhance revascularization are needed. Immunodeficient mice engrafted with ovarian tissue can serve as useful hosts for examining graft survival. *Host* treatment with melatonin with *graft* incubation with hyaluronan-rich biological glue+vascular endothelial growth factor A+ vitamin E (the "improvement protocol") promoted better human ovarian implantation in immunodeficient mice. SIS (Cook Biotechnology) enhanced wound healing and tissue remodeling also of rabbit ovaries, and Collage (Collplant) seems a promising new material for tissue repair.
Study design, size, duration: Frozen-thawed human ovarian samples were transplanted into 128 mice. Thawed tissue served as ungrafted control. Tissue was transplanted into the following groups: (1) untreated (grafted control); (2) with SIS or Collage alone; (3) with SIS or Collage+ "improvement protocol" *graft* treatment; (4) with SIS or Collage+ "improvement protocol" *host* treatment; (5) with SIS or Collage+"improvement protocol"; (6) with "improvement protocol" alone. The mice were euthanized 3 weeks after surgery, and the transplants were removed and fixed.
Participants/materials, setting, methods: Ovarian tissue was donated by nine women/girls (age 9–23 years) who underwent ovarian fertility cryopreservation in a tertiary medical center. Informed consent was obtained from the patients or parents of minors. Immunodeficient mice were engrafted with tissue covered with SIS or Collage with/without combinations of the "improvement protocol." Graft survival was assessed by follicle counts, apoptosis assay, immunohistochemical studies of Ki67 (follicular proliferation) and of platelet endothelial cell adhesion molecule (PECAM) (neovascularization) expression.
Main results and the role of chance: Collage and SIS fused well with the grafted ovarian tissue and neovascularization (PECAM staining) was identified in both Collage and SIS. Tissue apoptosis levels were significantly higher with Collage+the "improvement protocol" with/without *host* treatment (p = 0.03, 0.0015, respectively) than with Collage alone or the "improvement protocol" alone. Tissue apoptosis levels were significantly higher in all groups grafted with SIS with/without combinations of the "improvement protocol" than with the "improvement protocol" alone (p = 0.0025–0.0003). Follicular numbers were higher in ungrafted controls, but differences were statistically significant only compared to groups transplanted with SIS alone (p = 0.003) and SIS+ "improvement protocol" *host* treatment (p = 0.02). In grafted samples, recovered follicle number was highest in samples transplanted with Collage alone (NS) than in other groups. The number of atretic follicles was significantly high in most groups grafted with Collage combinations "improvement protocol" compared to Collage alone (p = 0.0002) or the "improvement protocol" alone (p = 0.03). The number of atretic follicles was significantly higher in most groups grafted with SIS with/without the "improvement protocol" than with the "improvement protocol" alone (p = 0.01 to <0.0001). Ki67 staining was identified in all morphologically normal grafted/ungrafted follicles.
Role of chance: There is an uneven ovarian follicular distribution, and we might have initially inadvertently transplanted slices with few follicles.
Limitations, reasons for caution: The availability of human ovaries for research is limited, and we could not allocate ovarian tissue from each patient in parallel to all groups. Moreover, although ovarian tissue from young girls contains numerous follicles their ovaries are small.
Wider implications of the findings: Results with Collage alone were at least as promising as those with the "improvement protocol" alone**.** Collage is non-allergic, non immunogenic and without potential risks of animal derived pathogens. Therefore, its use for grafting ovarian tissue may be easily applied for autotransplantation in cancer survivors without foreseeable risks.
Trial registration number: This not a clinical trial and a trial registration number is not required.

O-078 *In vitro* culture of human ovarian cortical strips in gas permeable dishes improves quality, viability and progression of follicles

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Study question: Does an increased oxygen perfusion through the use of gas permeable dishes affect the quality, viability and progression of follicles in cultured human ovarian cortical strips?
Summary answer: An increased oxygen supply beneath human ovarian cortical strips during *in vitro* culture better preserves the quality, viability and progression of primordial toward secondary follicles.
What is known already: Ovarian tissue culture is a broadly focussed topic in fertility preservation and follicle *in vitro* growth. Conventional cultures fail to support ovarian cortical tissue at the long term, mainly due to the fact that basic physical requirements like oxygen tension and other mechanical stimuli are neglected. In this view, occurrence of central necrosis due to inefficient oxygen and nutrient perfusion is a major hurdle in establishing long term ovarian cortical cultures.
Study design, size, duration: Ten 1 mm × 1 mm × 0.5 mm strips/dish (from the same ovary) were cultured for 9 days in α MEM with 0.1% BSA, 3mM glutamine, 50 µg/ml ascorbic acid, 1% ITS and 1% pen/strep in 5 ml in conventional (CD) and gas permeable (PD) 6 cm dishes in 5% CO^2 in air. Grading and staging of follicles was assessed through histology, and viability through live-dead far red at the confocal microscope.
Participants/materials, setting, methods: Ovarian cortical biopsies collected from three consenting patients (24, 27 and 30 years old) were transported in Leibovitz L15 at 4°C to the lab within 2 h, dissected into strips with a tissue chopper and cultured in CD and PD. Fresh and cultured strips at 6 and 9 days were fixed in Bouin for histological analysis or labelled with live-dead far red and Hoechst 33342 for assessment of viability at the confocal microscope.
Main results and the role of chance: Overall data was collected from 1784 follicles. Fresh tissue at day 0 had a high quality (grade: 1, 47.2; 2, 45.3; 3, 7.5%) and most follicles were at the primordial stage (primordial, 85.5; primary, 14; secondary, 0.5%). At day 6 strips cultured in PD had a higher quality, progression to the secondary stage and viability (grade: 1, 30; 2, 18, 3, 52%; primordial 38; primary, 57; secondary, 5%; viability, 58%) compared to CD (grade: 1, 0; 2, 14; 3, 86%; 1, $P < 0.001$; 2, NS; 3, $P < 0.001$; primordial 31; primary, 69; secondary, 0%, $P < 0.001$; viability, 35%, $P < 0.01$). Data at day 9 confirmed that PD was able to support a higher follicle quality and viability (grade: 1, 30; 2, 21, 3, 49%; viability, 70%) compared to CD (grade: 1, 0; 2, 16, 3, 84%; 1, $P < 0.001$; 2, NS; 3, $P < 0.001$; viability, 47%, $P < 0.01$). Although there was a trend of PD to support a higher progression to the secondary stages, it was not statistically significant (PD: primordial 43; primary, 55; secondary, 2%; CD: primordial 34.5; primary, 64; secondary, 1.5%).
Limitations, reasons for caution: As this is a preliminary study, findings should be confirmed on a higher number of patients.
Wider implications of the findings: Oxygen perfusion dynamics represents a key factor that can control both long term survival and progression of follicles cultured *in situ*. These findings, translated into the design of a continuous flow-bioreactor system that more closely mimics the dynamic ovarian environment *in vivo*, could be applied clinically.
Trial registration number: None.

O-079 Do viable human preantral follicles recover after enzymatic isolation and vitrification?

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Study question: How is the survival of preantral follicles after enzymatic isolation and subsequent vitrification linked to follicle diameter and morphology?
Summary answer: Enzymatically isolated preantral follicles of intact morphology can be vitrified in a closed system, while small follicles tend to have a lower survival rate.
What is known already: The technique of cryopreservation of ovarian tissue is well established in human fertility preservation, however, reintroduction of cancer cells during reimplantation is a major concern. Isolation of preantral follicles may overcome this problem either at the time of ovarian tissue prelevation

or after thawing. Vitrification of preantral follicles is well established in animal models but as far as we know has never been investigated in humans.
Study design, size, duration: Experimental study in an academic research unit using frozen- thawed ovarian tissue donated for research purposes. Preantral follicles were enzymatically isolated and stained with Neutral Red (N.R.) during isolation. After determining diameter and morphology, viable (N.R. positive) follicles were vitrified. After warming, the follicles were stained again with N.R. Follicles were grouped according to diameter and morphology and survival was compared between groups.
Participants/materials, setting, methods: Cortical ovarian tissue fragments of three former cancer patients, frozen between 2001 and 2003 using a slow freezing protocol, were thawed and digested in Liberase DH and DNase. Neutral Red was added as a viability stain. Isolated viable follicles were vitrified using Vit Kit Freeze and up to 8 follicles were loaded per HSV straw.
Main results and the role of chance: One hundred and fourteen isolated N.R. positive follicles were vitrified of which 101 (88.6%) were recovered after warming. Follicles were classified as being small (<50 µm, 31.6%), medium (50–70 µm, 47.4%) or large (>70 µm, 21.0%). Larger follicles had a higher recovery rate (95.8%) as opposed to 85.2% for medium sized follicles and 88.9% for small ones although these differences were not significant.

Overall, 61.4% of follicles survived vitrification. There was a tendency for a better viability after warming for medium and large follicles (69.6 and 65.2% respectively) compared to 46.9% for small follicles ($p > 0.05$). According to morphology, follicles were classified as being intact (intact basal membrane, close contact between granulosa cells and oocyte), fair (intact basal membrane and loosening of the contact between granulosa cells and oocyte) or compromised (damaged basal membrane). Intact follicles survived significantly better (90.9%) than fair (54.3%) and compromised (9.5%) follicles ($p < 0.005$).

Although potential damage by the use of cryopreserved ovarian tissue, isolation techniques and subsequent vitrification could occur, we still observed a good recovery rate of viable follicles.

Limitations, reasons for caution: Viability was assessed by one parameter only (N.R.) and no culture after thawing was performed to check for developmental competence.

Wider implications of the findings: Isolated human follicles can successfully be vitrified and stored for later use, even after having been cryopreserved for more than a decade. Further research into follicular development and matrix-carrier technology will broaden the horizon of fertility preservation.
Trial registration number: None.

O-080 Are there factors which predict the success of ovarian tissue grafting in oncofertility patients?

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Study question: For oncofertiltiy patients having ovarian tissue grafting what factors, either in the graft tissue or the maternal environment, are associated with longevity of ovarian function or fertility/pregnancy?
Summary answer: Age of patient at time of tissue excision and follicular density were most strongly correlated with graft survival and potential fertility.
What is known already: Ovarian tissue cryopreservation with subsequent grafting is increasingly recognised as a realistic option for fertility preservation in female patients with cancer or other serious disease requiring gonadotoxic therapy. It is the only option for prepubertal girls. However, development of good quality embryos and achievement of pregnancy is still disappointingly elusive, perhaps at least partly due to accelerated atresia of follicles in the grafted tissue. Technical modifications may improve graft function and pregnancy rates and many factors, related to tissue handling, preparation of the environment, and stimulation protocols, are being investigated.
Study design, size, duration: We performed 28 ovarian tissue grafts in 19 patents, average age 28 years (range 18–37) at time of excision and 37 years (range 25 to 45) at time of grafting. Follow up after grafting has been performed for 1–8 years. Nine patients have requested fertility assistance with IVF and a modified low-dose stimulation protocol was employed.
Participants/materials, setting, methods: Young women who had developed premature ovarian failure were assessed for suitability for grafting and all tissue was assessed to exclude tumour. Graft sites included ovarian bed, pelvic side wall and anterior abdominal wall with 7 grafts performed in all three locations, 16 in two locations and five in one location. Monitoring was performed for endocrine and biophysical evidence of restoration of ovarian activity and follicular development, and subsequent fertility assistance was performed with IVF.
Main results and the role of chance: Restoration of ovarian function was achieved in 17/19 patients. Surprisingly, chemotherapy did not correlate with graft activity or duration of function. Graft function commenced on average 4 months (range 1–9 months) after surgery and duration of function of 1–55 months (ongoing) has been demonstrated. Younger age at tissue excision ($r = –0.69$, $p = 0.4$) and follicle number ($r = 0.61$, $p = 0.5$) were most strongly correlated with graft survival. Three births and a further three biochemical pregnancies have been achieved from a total of 21 embryos transferred, with 7 frozen oocytes and 8 frozen embryos currently in storage. However no oocytes were retrieved in 26% of treatment cycles. The twin delivery from abdominal grafting in a patient who had previously had bilateral oophorectomy unequivocally excludes the possibility of a spontaneous pregnancy.
Limitations, reasons for caution: The small number of patients (although one of the largest series reported), incomplete data regarding follicular density and total volume grafted, and our inability to accurately gauge the extent of follicular atresia all hampered our ability to more rigorously investigate predictors of success of graft function.
Wider implications of the findings: A previous report (Schmidt et al., 2010) in a smaller study did not demonstrate a clear relationship between follicle number/volume and graft function. However increasing attention to methods cryopreservation and *in vitro* models of graft function will allow more comprehensive understanding of graft follicle dynamics and hopefully better fertility outcomes.
Trial registration number: Institutional Review Approval.

SELECTED ORAL COMMUNICATIONS

SESSION 21: POOR RESPONDERS: NEW HOPE?

Monday 04 July 2016 **Room 101** **15:15–16:30**

O-081 The impact of total follicle-stimulating hormone (FSH) dose on outcomes in ESHRE Bologna poor ovarian responders. A *post-hoc* analysis of the ESPART randomized controlled trial

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Study question: Does FSH dose influence outcomes for poor ovarian responders (PORs) undergoing controlled ovarian stimulation with recombinant-human FSH (r-hFSH) or r-hFSH plus recombinant-human luteinizing hormone (r-hLH)?
Summary answer: In the ESPART study, PORs receiving a total FSH dose of 3000 to < 5000 IU had better outcomes than those receiving < 3000 or ≥5000 IU doses.
What is known already: A large retrospective database analysis by Baker et al. (2015) in IVF patients, suggested that live birth rates significantly decreased with increasing FSH dose, except in women ≥35 years with 1–5 oocytes retrieved. The ESPART randomized controlled trial investigated assisted reproductive technologies (ART) outcomes following controlled ovarian stimulation with r-hFSH or r-hFSH+r-hLH in PORs who met ≥2 of the following criteria (aligned with the Bologna criteria): >40 to <41 years; previous ART cycle with ≤3 oocytes retrieved with conventional stimulation; AMH level 0.12–1.3 ng/mL. A total of 939 patients were randomized and no difference in outcomes was observed between treatments.
Study design, size, duration: This was a *post-hoc* analysis of the ESPART study, not including patients with cycle cancellations. Number of oocytes retrieved, ongoing pregnancy rate (OPR), and live birth rate (LBR) over a single ART cycle were determined for patients grouped according to the total FSH dose received with both treatments. The dose groups investigated were: <3000 IU, 3000 to <4000 IU, 4000 to <5000 IU and ≥5000 IU, and data were summarized descriptively. Individual patient-level data were also investigated.

Participants/materials, setting, methods: The modified intention-to-treat population from the ESPART study, excluding patients with cycle cancellations, (r-hFSH+r-hLH, *n* = 427; r-hFSH, *n* = 445) was analysed. The mean [standard deviation (SD)] age of patients was 38.3 (3.0) years and the mean (SD) number of oocytes retrieved was 3.3 (2.71) and 3.6 (2.82) in patients receiving r-hFSH+r-hLH and r-hFSH, respectively. With r-hFSH+r-hLH, there were 51/462 (11.0%) and 49/462 (10.6%) ongoing pregnancies and live births, respectively; with r-hFSH, 59/477 (12.4%) and 56/477 (11.7%), respectively.
Main results and the role of chance: The total mean FSH dose was similar in both arms**.** The highest number of oocytes retrieved, OPR and LBR was observed with total FSH doses of 3000 to < 5000 IU, and results were similar for both treatment arms. When FSH dose <3000 or ≥5000 IU was used, the number of oocytes retrieved was lower, but similar in both treatment arms. There was a different trend in OPR and LBR at FSH <3000 and ≥5000 IU with or without LH supplementation to FSH (**Table**).

FSH dose	<3000 IU	3000 to <4000 IU	4000 to <5000 IU	≥5000 IU
Age (years), mean (SD)	37.9 (3.2) (*N* = 115)	38.3 (3.0) (*N* = 381)	38.5 (2.8) (*N* = 268)	38.2 (2.9) (*N* = 175)
AMH (ng/mL), mean (SD)	0.52 (0.39) (*N* = 114)	0.69 (0.53) (*N* = 379)	0.61 (0.51) (*N* = 266)	0.40 (0.33) (*N* = 174)
Number of oocytes retrieved, mean (SD)				
r-hFSH	3.3 (2.38) (*N* = 34)	4.4 (3.15) (*N* = 183)	3.7 (2.31) (*N* = 141)	2.9 (2.30) (*N* = 87)
r-hFSH+r-hLH	3.3 (2.29) (*N* = 61)	4.2 (2.97) (*N* = 179)	3.5 (2.32) (*N* = 111)	2.7 (2.15) (*N* = 76)
Ongoing pregnancy, *n*/*N* (%)				
r-hFSH	2/34 (5.9)	29/183 (15.8)	17/141 (12.1)	10/87 (11.5)
r-hFSH+r-hLH	7/61 (11.5)	27/179 (15.1)	15/111 (13.5)	3/76 (3.9)
Live birth, *n*/*N* (%)				
r-hFSH	2/34 (5.9)	28/183 (15.3)	16/141 (11.3)	9/87 (10.3)
r-hFSH+r-hLH	7/61 (11.5)	26/179 (14.5)	14/111 (12.6)	3/76 (3.9)

Limitations, reasons for caution: This was a *post-hoc* analysis, and the results should, therefore, be treated as exploratory. The inclusion criteria were aligned with the Bologna criteria, but not identical to them. Therefore, these results may not be applicable to the wider POR population.
Wider implications of the findings**:** This suggests PORs have maximal responses with 3000 to <5000 IU FSH. Outcomes for PORs not responding to these doses are unlikely to improve at higher doses, while lower doses seem insufficient for maximal response. The different pregnancy and live-birth outcomes observed at <3000 and ≥5000 IU with/without LH supplementation warrants further investigation.
Trial registration number: ClinicalTrials.gov identifier: NCT02047227; EudraCT Number: 2013-003817-16.

O-082 A randomised double blind placebo controlled study of recombinant human growth hormone (r-HGH) on live birth rates in women who are poor responders

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Study question: The primary objective of the study is the live birth rate in poor IVF responders.
Summary answer: This study showed no difference in the live birth rate between the r-HGH or placebo controlled group.
What is known already: Human Growth Hormone (HGH) is important for ovarian steroidogenesis and follicular development. Research to date indicates a trend to an improved live birth rate using HGH as an adjunct in an IVF cycle. However these studies have been underpowered, have significant clinical heterogeneity and the safety outcomes are uncertain.
Study design, size, duration: The study required an enrolment of 390 women to be recruited from 9 fertility centres in Australia and 1 centre in New Zealand. Allocation concealment was ensured by using prenumbered study drug kits allocated to participants on day 1 of their IVF cycle. The group allocation relating to this number was blinded by clinicians and participants until study completion. It was expected this study would be completed in 2 years.
Participants/materials, setting, methods: One hundred and thirty six participants, defined as well women who had a previous IVF/ICSI cycle with >250 IU FSH and <5 oocytes collected, an FSH level <15 IU were recruited to the study. Recombinant FSH stimulation >250 IU, started on day 2/3, study drug commenced on the same day as r-HGH injections All cycles required the use of an antagonist. Cycle management was according to each centres standard protocol.
Main results and the role of chance: One hundred and thirty one participants were randomized, 66 to the r-HGH group and 65 to placebo. 116 had oocyte retrieval, 97 had embryo transfer. 25 participants reported a positive pregnancy test, 16 delivered a live birth. This included 3 sets of twins, all in the r-HGH group. The overall live birth rate per patient randomized = 12.3%. Four serious adverse events, all congenital abnormalities, occurred, 3 in the r-HGH group and 1 in the placebo group. No serious adverse events occurred in the women.

	r-hGH	**Placebo**
Clinical pregnancy (%)	9.1	9.2
No. of oocytes collected (mean)	5.38 (SD 3.43)	4.96 (SD 3.47)
Embryo quality (median)	2.00 (1.00, 3.67)	2.14 (1.00, 4.00)
Days of FSH stimulation (median)	8 (2, 13)	8 (2, 15)
Days of a study drug (median)	8 (7, 14)	9 (0, 15)
No. of embryos transferred (mean)	1.0 (0, 2.0)	1.0 (0, 2.0)

Limitations, reasons for caution: Recruitment proved very difficult and the study was terminated after 4 years due to slow recruitment. The original numbers for enrolment were not reached; hence our study remained underpowered to answer our question. These results are based on an intention to treat analysis.

Wider implications of the findings: This study highlights the difficulty in answering important questions to provide the evidence to enable the best clinical treatment**.** The literature to date is diverse and divisive. Much of the literature does not address the most important clinical outcome our patients require, that of delivering a live healthy infant.
Trial registration number: ACTRN12609001060235 Australian New Zealand Clinical Trial Registry.

O-083 A prospective randomized controlled study depicting favourable IVF outcomes of pretreatment with transdermal-testosterone in poor-responders undergoing ART cycles

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Study question: To evaluate the effect of transdermal testosterone preceding ovarian stimulation, in improving IVF outcome in women with poor ovarian response undergoing IVF-ICSI cycles?
Summary answer: Pretreatment with Transdermal testosterone may improve ovarian response to gonadotrophins in low-responder IVF patients.
What is known already: The poor response to ovarian stimulation among women undergoing IVF is of great concern in reproductive medicine. The reported incidence of poor response varies from 10 to 30%. Certain modalities have been tested to improve the response to gonadotrophin stimulation and thus reproductive outcomes. In daily practice, the most commonly used agents before or during ovarian stimulation are transdermal testosterone, dehydroepiandrosterone (DHEA), aromatase inhibitors, recombinant LH and recombinant human chorionic gonadotrophin (HCG). The results from previous many trials that aim to increase intra ovarian androgen concentrations in poor responders have shown conflicting results.
Study design, size, duration: Prospective randomized controlled trial to investigate the effectiveness of treatment with transdermal testosterone gel before controlled ovarian stimulation in 214 low responders patients between 2010 and 2015.

Our objective was to determine if administration of exogenous testosterone prior to gonadotropin stimulation leads to better IVF-ICSI outcome in known poor responders.

There were no differences in patients' characteristics between the two groups.

Patients were randomized into TTG pretreatment group and control group.
Participants/materials, setting, methods: A total of 214 poor responders (2010–2015) were included, defined as those who failed to produce <3 follicles with the result that <3 oocytes were retrieved despite the use of a high gonadotropin dose, in a previous failed IVF cycle. Patients were randomized into TTG pretreatment group and control group. For TTG pretreatment group, 12.5 mg TTG was applied daily for 21 days in the cycle preceding IVF cycle. GnRH antagonist protocol was used in both groups.
Main results and the role of chance: Total dose and days of FSH used were significantly fewer in the TTG pretreatment group than in the control group. The numbers of oocytes retrieved, mature oocytes, fertilized oocytes, and good-quality embryos were significantly higher in the TTG pretreatment group. Embryo implantation rate and clinical pregnancy rate per cycle initiated also were significantly higher in the women pretreated with TTG.

The primary outcome measured was the number of mature oocytes retrieved. Secondary outcomes included total amount and days of FSH administered, numbers of fertilized oocytes and good-quality embryos, implantation rate, clinical pregnancy rate per cycle, and live birth rate per cycle. Embryo implantation rate (13%) was significantly higher in the TTG pretreatment group than in the control group (06%). The clinical pregnancy rates were also significantly higher in the TTG pretreatment group than in the control group (29 and 14% respectively).
Limitations, reasons for caution: Studies to date have focused on older patients or women with reduced ovarian reserve and "resurrecting" these ovaries may be an impossible task. Considering the relatively long duration of folliculogenesis compared with exposure to androgens used, possibly different doses and duration of androgen treatment may ultimately be required.
Wider implications of the findings: TTG pretreatment might be beneficial in improving both response to COS and IVF outcome in poor responders undergoing IVF/ICSI. Our study demonstrated that TTG pretreatment can increase the numbers of oocytes retrieved, fertilized oocytes, better embryos and pregnancy rate as well as reduce the amount of FSH required during IVF/ICSI cycles.
Trial registration number: BTTBC/2010/13.

O-084 Dehydroepiandrosterone administration does not increase pregnancy rates in poor responders: a meta-analysis

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Study question: Does dehydroepiandrosterone (DHEA) administration improve the probability of pregnancy in poor responders undergoing ovarian stimulation for *in vitro* fertilization (IVF)?
Summary answer: DHEA administration in poor responders undergoing ovarian stimulation for IVF is not associated with an increase in the probability of pregnancy.
What is known already: DHEA administration in poor responders undergoing ovarian stimulation for IVF has been hypothesised to increase the intra-ovarian androgen concentration and the sensitivity of developing follicles to follicle stimulating hormone. A recently published Cochrane meta-analysis suggests that treatment with DHEA is associated with an improved probability of pregnancy in poor responders. However, this conclusion was based on synthesis of data from studies that did not recruit exclusively poor responders and thus it is of questionable value.
Study design, size, duration: A literature search was performed in MEDLINE, CENTRAL and Web of Science until January 2016, aiming to identify randomised controlled trials (RCTs) evaluating DHEA administration exclusively in poor responders undergoing IVF. Seven eligible RCTs evaluating a total of 576 patients were meta-analysed.
Participants/materials, setting, methods: The main outcome measure was achievement of pregnancy, expressed as either clinical pregnancy or as live birth. Secondary outcome measures included duration of stimulation, total units of gonadotrophins required, number of cumulus-oocyte complexes (COCs) retrieved, number of 2-pronuclei oocytes and number of embryos transferred.
Main results and the role of chance: Administration of DHEA for a period of 6–12 weeks prior to ovarian stimulation did not improve significantly the probability of clinical pregnancy (RR: 1.10; 95% CI: 0.81–1.50) or live birth (RR: 1.18; 95% CI: 0.36–3.88) as compared to no DHEA treatment. Although DHEA administration was associated with a significant decrease in the total units of gonadotropin required (WMD: −783.9 IU, 95% CI: −1065.6 to −502.3), no significant differences were observed in the duration of stimulation (WMD: −1.25 days, 95% CI: −2.53 to +0.03), in the number of COCs retrieved (WMD: +0.66 COCs, 95% CI: −1.88 to +3.19), in the number of 2-pronuclei oocytes (WMD: +1.13 2pn, 95% CI: −0.32 to +2.58) and in the number of embryos transferred (WMD: +0.53 embryos, 95% CI: −0.24 to +1.30), respectively.
Limitations, reasons for caution: The definition of poor ovarian response, the protocols used for DHEA administration and for ovarian stimulation varied among studies. Despite the fact that statistical heterogeneity was managed by using random effects models, where appropriate, the limited number of the studies analysed precluded an insightful evaluation of clinical heterogeneity.
Wider implications of the findings: The current meta-analysis, based on synthesis of data from RCTs including exclusively poor responders, does not suggest that DHEA improves the probability of pregnancy in this category of patients. This information is important for appropriately counselling poor responders without offering them unrealistic expectations regarding their prognosis.
Trial registration number: –.

O-085 Elevated progesterone on the day of triggering final oocyte maturation significantly predicts its reoccurrence in a subsequent IVF cycle

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Study question: Does progesterone elevation (PE) on the day of triggering final oocyte maturation during an IVF cycle increase the risk of PE in subsequent cycles?
Summary answer: History of PE on the day of triggering final oocyte maturation is a significant predictor of its reoccurrence in a subsequent IVF cycle.
What is known already: Progesterone elevation on the day of triggering final oocyte maturation is associated with the magnitude of the ovarian response to stimulation. Whether patients with previous occurrences of PE on the day of hCG are at a higher risk for PE in subsequent cycles as compared to patients with no PE in a previous cycle is not currently known.
Study design, size, duration: This is a retrospective analysis of a cohort of fresh IVF/ICSI cycles (N = 1,702) performed in a single IVF center during the period 2001–2015.
Participants/materials, setting, methods: Patients in which ovarian stimulation was performed with FSH and GnRH antagonists and with basal follicle FSH <14.0 mIU/mL and estradiol (E2) ≤80 pg/mL prior to initiation of stimulation were examined. Cycles from patients that had contributed ≥2 cycles (n = 197) in the study sample were analyzed by generalized estimating equation (GEE) analysis to calculate odds ratio (OR) with 95% confidence intervals (CI) for the prediction of PE occurrence and also to adjust for the intensity of ovarian stimulation.
Main results and the role of chance: Overall, 168 patients contributed 2 cycles, 25 patients contributed 3 cycles and 4 patients contributed 4 cycles. In 230 cycles (with a previous cycle in the study sample), 15 exhibited progesterone elevation (6.5%, 95% CI: 4.0–10.5) while in 22 cycles there was history of PE in a prior cycle (9.6%, 95% CI: 6.4–14.1). In six out of these 22 cycles, PE reoccurred, while this was the case only in 9 out of the 208 cycles without a history of PE.

A GEE regression analysis indicated that PE on the day of hCG in a previous cycle was associated with an increased risk of PE in a subsequent cycle (OR: 8.4, 95% CI: 2.8–24.9). The adjusted risk of PE was 4.3% (95% CI: 1.5–7.0) and 27.3% (95% CI 10.3–44.3) for cycles without and with history of PE, respectively. A full model adjusting for the intensity of ovarian stimulation showed that history of PE was still a significant predictor (OR: 6.26, 95% CI: 1.81–21.62).
Limitations, reasons for caution: This is a retrospective analysis and although the effect of the most important confounders was controlled for in the multivariable analysis, the presence of residual bias cannot be excluded.
Wider implications of the findings: The findings of this study might help clinicians identify patients at high risk for PE and either opt for an approach that will reduce the probability of PE (e.g., milder stimulation) or counsel them

about the increased probability of freezing all embryos and deferring embryo transfer.

Trial registration number: Not applicable.

INVITED SESSION

SESSION 22: THE NEW GENETICS FRONTIER: UP CLOSE AND PERSONAL

Monday 04 July 2016 **Hall 5 CB** **17:00–18:00**

O-086 Extensive sequencing of the embryo: implications and complications

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Single-cell genomics enables investigating the extent and nature of genomic and transcriptomic heterogeneity which occurs in both normal development and disease, and provides new tools for clinical application. We have developed various *in vitro* and *in silico* methods that allow analysing a solitary cell at high resolution via microarray and next-generation sequencing platforms. Most recently, we developed methods to sequence both DNA and RNA of the same single cell, enabling genotype-phenotype correlations on the single-cell level (Macaulay et al., 2015; Angermueller et al., 2016), as well as methods for concurrent DNA copy number typing and haplotyping of single cells (Zamani Esteki et al., 2015). Data on the application of these methods for understanding the biology of cellular heterogeneity during the first cell divisions of life will be presented (Bolton et al., 2016; Goolam et al., 2016; Destouni et al., in press), including also unpublished data on human preimplantation embryos. Finally, novel single-cell genome-wide analysis methods that are now clinically applied for human embryo selection for the purpose of preimplantation genetic diagnosis (Zamani Esteki et al., 2015) and a visionary on the future will be introduced.

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O-087 Genomics and you: predicting lifetime disease risk

X. Estivill[1]

[1]*Center for Genomic Regulation (CRG), Genomics and Disease, Barcelona, Spain*

INVITED SESSION

SESSION 23: REPRODUCTION AND RHYTHMICITY

Monday 04 July 2016 **Hall 5 A** **17:00–18:00**

O-088 Timing in reproduction in mammals by photoperiod

F.J.P. Ebling[1]

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Seasonal reproduction is the norm amongst mammals indigenous to temperate and polar regions, such that offspring are born in the optimal season for their mothers to support pregnancy and lactation, and for their own survival. Almost all species use the annual change in photoperiod as the primary cue to regulate the seasonal changes in neuroendocrine function that underlie such reproductive rhythms. In the last decade huge advances have been made in understanding the mechanisms whereby this happens, revealing neuroendocrine pathways that may help us understand hypothalamic control of energy metabolism and reproduction in humans. Annual changes in daylength are primarily detected by melanopsin-containing retinal ganglion cells. This information is integrated by the suprachiasmatic nucleus of the hypothalamus, and regulates the nocturnal duration of melatonin secretion. Although the pivotal role of the changing pattern of melatonin secretion in seasonal reproduction has long been known in mammals, recent evidence indicates that the pars tuberalis (pituitary stalk) is a key site of action of melatonin. The short duration of nocturnal melatonin secretion in long days promotes thyroid stimulating hormone (βTSH) secretion from the *pars tuberalis*, which acts in paracrine fashion on hypothalamic tanycytes whose end feet project to the pituitary stalk. βTSH promotes expression of deiodinase 2 gene in tanycytes, which catalyses the local conversion of inactive thyroid hormone (thyroxine: T_4) into the active form of thyroid hormone: T_3 (tri-iodothyronine). Conversely, in short days, the long nocturnal duration of melatonin reduces the βTSH signal from the *pars tuberalis,* and deiodinase 2 gene expression decreases in tanycytes, whereas deiodinase 3 (DIO3) gene expression increases. DIO3 deiodinates T_4 and T_3 to generate inactive compounds, so across all seasonal mammals studied to date there is a clear photoperiod-regulated change in local availability of T_3 in the hypothalamus. The increase in local hypothalamic T_3 concentrations in long days and the consequent decrease in short days are functionally important for activity of the hypothalamic–pituitary–gonadal axis. Our experiments in the Siberian hamster have revealed that placement of microimplants releasing T_3 into the hypothalamus blocks short-day induced testicular regression. Correspondingly, placement of such implants into animals previously exposed to short days with regressed testes will induce gonadal recrudescence. Thus, the high T_3 concentrations in long days promote reproductive activity, but the reduction of T_3 in short days results in the inactivation of the reproductive axis. These seasonal changes ultimately reflect changes in kisspeptin activation of gonadotrophin-releasing hormone neurons, but these neuroendocrine changes are not simply a direct consequence of high hypothalamic thyroid hormone concentrations. In seasonal species like sheep that become reproductively active under short days, the activation of the reproductive axis is associated with a decrease in hypothalamic T_3 concentrations, and an increase in T_3 in the hypothalamus is necessary to terminate the breeding season. As T_3 is critical for initial development of the brain in the fetus, it has been hypothesized that T_3 exerts long-term control of hypothalamic function by the recapitulation of mechanisms important in initial development. Tanycytes are a stem cell niche supporting hypothalamic neurogenesis in the adult brain, and play a key role in the transport and regulation of thyroid hormone availability and other developmentally important signals such as retinoic acid in the hypothalamus. According to this view, the hypothalamus as a plastic region of the brain, capable of being reprogrammed, so we should appreciate that seasonal species provide valuable model systems to elucidate the mechanisms underlying adult brain plasticity. Such plasticity is not only core to seasonal cycles of reproductive activity, but also to seasonal cycles of appetite, energy expenditure and fat metabolism, with which reproductive cycles are intimately associated.

O-089 Influences of environmental conditions on the reproductive system and fertility in humans

A. Lerchl[1]

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Most environmental factors fluctuate with considerable amplitude, such as temperature, photoperiod, tide, humidity and precipitation. Since all of them are a consequence of astronomical cycles of the earth, and the moon, respectively, they occur *cum granum sale* with high reliability and precision. Secondary to these changes, the presence of organisms at various times of the day and the year fluctuates with high precision and predictability as well. Therefore it is highly advantageous for a species' survival to adapt to such physical and biological environmental fluctuations with high reliability. In contrast to passively reacting to those changes, anticipating them is favorable, and therefore reliable biological clocks have evolved with frequencies of approximately

24 h (circadian), 12.5 h (circatidal), 28 days (circalunar), and 1 year (circannual). These rhythms persist without any environmental entraining rhythm ("zeitgeber"), and some of them are present even in unicellular organisms. In many mammalian species the time of reproduction is strictly regulated by the environmental factor photoperiod *via* the hormone melatonin and limited to a distinct period of the year, thus ensuring that the offspring find optimal conditions for survival after birth. Due to the different gestational length, rodents reproduce mainly in late spring and summer (long-day breeder), while ruminants are sexually active in late autumn or winter (short-day breeder). While humans are generally believed to reproduce throughout the year without a strict seasonality, there are nevertheless marked seasonal birth rate fluctuations which depend on geographical and secular influences. Because humans lack melatonin receptors in the *pars tuberalis* which is the site of action of the hormone in seasonal breeders, these fluctuations are considered to be potential epiphenomena of other factors, e.g., behavioral changes. This assumption is corroborated by observations showing a marked shift by 6 months in seasonal birth rates in many countries in the 1960s to 1980s. The explanation for this effect may be that environmental cues are more and more shielded from humans due to central heating and artificial illumination. Less easily explained are seasonal patterns of secondary sex ratios in humans and their influences by environmental temperatures before conception. Although these data are in good accordance with studies in rodents and other species, and although the effect as such makes sense in evolutionary terms, the mechanisms which are responsible for this effect are enigmatic. For the production of sperm, circadian rhythms may play a decisive role because a number of so-called clock genes have been found to be expressed at highly variable levels. It is argued that not only the maturation of sperm and oocytes depend on intact circadian clock genes, but also that the synthesis of steroids in the Leydig cells, and the granulosa cells, respectively, are severely hampered if the circadian gene expression machinery is not working properly. This kind of research is certainly promising in an area where a large proportion of infertility is diagnosed as idiopathic. In recent years, some attention has been attracted by studies allegedly showing detrimental effects of exposure to artificial radiofrequency electromagnetic fields (RF-EMF, such as those originating from mobile phones) on sperm production. While a possible mechanism for such effects has not been identified yet and is quite unlikely to exist for biophysical reasons, many experimental studies performed so far chronically suffer from inadequate exposure conditions. In contrast, carefully designed multi-generation studies in mice have not indicated any negative effect of RF-EMF exposure on both sexes. Likewise, the numbers of offspring up to the fourth generation were not affected.

INVITED SESSION

SESSION 24: CONSEQUENCES OF AN EXTRA X-CHROMOSOME – LESSONS FROM MOUSE MODELS AND CONSEQUENCES FOR CHILDREN

Monday 04 July 2016 **Hall 3 AB** **17:00–18:00**

O-090 Molecular mechanisms of the supernumerary X chromosome in the XXY* mouse model

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Study question: Can general molecular mechanisms and the genetic impact of the supernumerary X-chromosome be adequately examined in 41,XX^{Y}* male mice?
Summary answer: Data obtained in 41,XX^{Y}* mice suggest their usefulness for the examination of the effects of a supernumerary X-chromosome in a male environment.
What is known already: Klinefelter syndrome (KS, 47,XX^{Y}) provokes infertility, hypogonadism, gynecomastia, disturbed bone metabolism, diabetes, cardiovascular and cognitive problems. Although morbidity and mortality are increased, the disorder is underdiagnosed, likely because of heterogeneous phenotype and variable severity. Generally, loss of germ cells and hypogonadism are observed. Although X-inactivation is normal in KS men, genes escaping from silencing ("escapees") seem to provoke genetic and epigenetic changes resulting in affected DNA methylation and differentially expressed X-chromosomal and autosomal genes. However, clinical studies are limited. Thus, the availability of mouse models resembling human KS is of advantage.
Study design, size, duration: During the last 10 years we characterized 41,XX^{Y}* mice in several studies for inactivation of X-chromosomal genes, the dynamics of germ cell loss, testicular vascularization, and the function of the somatic Leydig and Sertoli cells as well as memory recognition over the entire postnatal life from newborn to adult animals. Male and female littermates of 41,XX^{Y}* mice served as controls.
Material/settings/methods: Male 41,XX^{Y}* and male and female control mice for experimental use were obtained from our B6Ei.Lt-Y* mouse colony. Group sizes varied between three animals per group and time point up to >40, dependent on the analyses. Expression of genes was examined by qPCR, protein abundances by immunohistochemistry, for endocrine measurements radioimmuno assays were employed, vascularization was assessed by ultrasound imaging and cognition by behavioural phenotyping.
Main results and the role of chance: In mice only few genes escape from X-inactivation making the analysis feasible compared to the more complex situation in patients. However, these few most relevant escapee genes which mice and men share, i.e., *Utx*, *Kdm5c*, *Eif2s3x* and *Ddx3x* are also inducing a phenotype very similar to human KS. Analyzing the expression of these genes in 41,XX^{Y}* male mice, we found tissue- and gene- but also development-specific expression profiles rendering genotype-phenotype relations more complex than thought so far. Physiologically, data from mouse models elucidated that the disturbed metabolism and the cognitive deficits have a genetic background and are not only metabolic consequences of the disorder. In addition, experimental exploration using the animals revealed germ cell loss to start already during the intrauterine life period and to be accompanied by fading expression of spermatogonial markers like Lin28 and Pgp9.5 in the early postnatal phase. However, the steroidogenic Leydig cells seem to function normally although being hyperactivated, i.e., not to be causative for hypogonadism, a finding which was later confirmed in patients. The latter finding pointed to a possible problem of testicular blood supply, confirmed recently by reporting the testicular vascularization in 41, XX^{Y}* mice to be disturbed.
Limitations, reasons for caution: Whilst human KS results in a heterogeneous phenotype; in the models it is homogeneous. A substantial proportion of KS patients exhibit XY/XX^{Y} mosaicism, not observed in the mice. Studies addressing the germ cell loss must consider different rodent and primate spermatogonial systems as well as developmental differences.
Wider implications of the findings: Experimental exploration of KS has identified genetic and epigenetic effects in KS which appear rather complex. Mouse models can be used to define genotype-phenotype correlates. This might become especially important in terms of so far overlooked features of the syndrome, e.g., the hampered circulation and cardiovascular problems.
Study funding: Deutsche Forschungsgemeinschaft; grants no. WI 2723/2-1, -4-1.

O-091 Treatment options for infertility in Klinefelter patients

H. Tournaye[1]
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At the onset of puberty, Klinefelter men face testicular stem cell loss by slowing-down their self-renewal and by intensified apoptosis. While in the adult Klinefelter testicular tubules show a Sertoli-cell-only pattern with sclerosis and fibrosis, occasional tubules showing active spermatogenesis may be observed in about half of them. In case, spermatozoa can be surgically harvested for ICSI. While in many case-series chromosomally normal offspring has been reported, at present it remains unclear how successful this strategy can be overall since these reports tend to overestimate the actual outcome in a biased way. There is also great controversy in regards to whether pubertal adolescent Klinefelter boys should have TESE offered to preserve future fertility. The controversy is even greater for fertility preservation in pre-pubertal Klinefelter boys. At present, there is no data demonstrating any benefit of early fertility preservation in adolescents compared to the adult patients. No data at all exist on the benefit of testicular tissue banking at pre-pubertal age. Testicular tissue freezing in pre-pubertal 47,XX^{Y} boys requires further validation in a research framework. Not only do we need to scrutinize the potential benefits vs. drawbacks, but because of progressing fibrosis of the testes, the need for *in vitro* maturation strategies should be investigated too.

SELECTED ORAL COMMUNICATIONS

SESSION 25: PARAMEDICAL 2 - LABORATORY

Monday 04 July 2016 **Room 101** **17:00–18:00**

O-092 Effect of the timing of oocyte insemination or injection on the fertilization and embryo utilization rates in assisted reproduction

B. Desmet[1], S. Santos-Ribeiro[1], N. De Munck[1], W. Meul[1], H. Van de Velde[1], G. Verheyen[1]
[1]*UZ Brussel, Centre for Reproductive Medicine, Jette, Brussels, Belgium*

Study question: Does the time interval between ovulation triggering and oocyte insemination or injection affect the fertilization and utilization rates in conventional IVF and ICSI cycles?
Summary answer: There is no clinically relevant impact of oocyte insemination or injection timing after ovulation triggering on fertilization and embryo utilization rates.
What is known already: Oocyte retrievals are commonly scheduled 36 h after ovulation triggering. Previous studies have shown that MII oocyte nuclear and cytoplasmic maturation seems to be optimal 38 h to 39 h after ovulation triggering, which corresponds with 2 to 3 h post-retrieval. Time intervals above 40 h may lead to *in vitro* oocyte aging and an increased risk of ultrastructural defects. However, it may be difficult from a practical point of view to respect this short timeframe, especially in ART centres with heavy workloads. Time intervals lower than 38 h and higher than 39 h are common practice in busy IVF laboratories.
Study design, size, duration: A single-centre retrospective cohort analysis of 1696 IVF and 14,553 ICSI cycles from 2008 until 2015 was performed. The time between ovulation triggering and oocyte insemination/injection were grouped in one of the 7 following regular 1 h-interval categories: <36, 36, 37, 38, 39, 40 and ≥41 h. Our main outcome measures were the fertilization and utilization rates (embryos adequate for transfer or cryopreservation) per inseminated cumulus-oocyte complex for IVF or per MII for ICSI.
Participants/materials, setting, methods: Only cycles using fresh autologous gametes were included. We excluded managed natural cycles, cycles with combined conventional IVF/ICSI, PGD cycles and IVM cycles. In total, we analyzed 16,866 inseminated (IVF) and 104,593 injected (ICSI) oocytes. We performed mixed-effects multilevel multivariable regression analysis, accounting for the clustering of oocytes by cycle and patient and also the following potential confounders: female age, pre-preparation sperm concentration and post-preparation sperm motility.
Main results and the role of chance: In the IVF group, no significant differences in the adjusted fertilization (range 57.4–62.9%) and utilization rates (range 30.4–34.8%) were observed between the time intervals. In the ICSI group, the pairwise comparison showed that, compared to all other timeframes, the adjusted fertilization rate was slightly but significantly lower for the <36 h interval (71.0 versus 78.3, 77.6, 78.0, 78.9, 79.3 and 79.9% for each 36 to > 41 h 1 h-regular interval, respectively). However, the adjusted utilization rates after ICSI did not vary significantly between all the time intervals assessed (41.1, 44.3, 43.1, 45.5, 44.8, 43.9 and 43.8%, respectively).
Limitations, reasons for caution: This was a retrospective study with its inherent limitations. Furthermore, these results should not be extrapolated to other cycle outcomes, such as pregnancy rates.
Wider implications of the findings: These results offer reassurance to centres which find themselves frequently forced to re-schedule oocyte insemination/injection timing owing to an extenuating workload. Although we found a slight decrease in fertilization after ICSI performed <36 h after triggering, this difference seemed clinically irrelevant since it had no expression on the utilization rates.
Trial registration number: None.

O-093 Cumulus growth pattern of *in vitro* matured cumulus-oocyte-complexes (COC) of transsexual persons obtained during cryopreservation of ovarian tissue correlates with the maturation status.

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[3]*University Hospital Ghent, Department of Endocrinology, Ghent, Belgium*

Study question: Is the morphology growth pattern of COCs from female-to-male transsexual persons during *in vitro* maturation (IVM) an indicator for oocyte meiotic competence?
Summary answer: Cumulus expansion was a significant positive indicator and the cumulus–oocyte interaction a significant negative indicator for maturity of the *in vitro* matured oocyte.
What is known already: Baboon oocytes collected from small antral follicles during ovary dissection in the luteal phase were *in-vitro* matured and produced viable embryos (Woodruff et al., 2011). The meiotic competence of these oocytes was positively correlated with the number of surrounding cumulus cells (CC). In PCOS patients, immature oocytes collected from non-stimulated cycles showed that the amount, the morphology of the CC and its connection to the oocyte played an important role in predicting the final quality of the COCs (Liu et al., 2010).
Study design, size, duration: Cumulus mass (CM); cumulus expansion (CE) and contact between CC and the oocyte (CO) were scored according to Smitz (2004), the size of the expansion was measured and calculated on day 0, 1, and 2. After 48 h IVM, oocytes were denudated and the maturation status was assessed.
Participants/materials, setting, methods: A total of 123 COCs were collected at the moment of cryopreservation of ovarian tissue from 4 female-to-male transgender persons with a mean age of 20.1 ± 2.7 years. IVM was performed for 48 h using a commercial IVM media (Origio). To assess the independent effect of each parameter, logistic linear regression analysis (SPSS v23) was performed to predict which variable was statistically associated with maturity ($P < 0.05$).
Main results and the role of chance: On day 0, 65.0% (80/123) of the COC were fully enclosed by their CC (CO), 87.8% (108/123) showed tight dense CC mass (CE) and 56.1% (69/123) had 10 or more layers of CC (CM). Generally, 43.1% (53/123) of the COCs were fully enclosed with tight dense cells of 10 or more layers of CC. Mean size of day 0 COCs was 366.5 ± 263.7 µm.

On day 1, 63.4% (78/123) of the COCs were still fully enclosed (CO). For the expansion status (CE), 35.8% (44/123) of the COCs showed already moderate expansion of their CC and 9.8% (12/123) showed fully expanded CC. After 24 h *in-vitro* culture, 5.7% (6/123) were already naked oocytes. Mean size of day 1 COCs was 473.9 ± 263.7 µm.

On day 2, 33.3% (41/123) of the COCs showed moderate expansion, 13.8% (17/123) showed fully expanded CC. After 48 h *in-vitro* culture, 22.0% (27/123) were naked oocytes.

Finally, 20.3% (25/123) of the IVM COCs reached MII stage. Statistical analysis showed that the CE on day 2 was a positive indicator for MII ($P = 0.021$) and that de CO was a negatively associated with MII ($P = 0.007$).
Limitations, reasons for caution: This study is limited in the number of COCs and patients used to conclude that morphological parameters can be clinically applied as statistical prognostic tool in the oocyte maturation outcome in IVM programs for transgender persons.
Wider implications of the findings: The morphological growth pattern during maturation can help us to study the maturation competence of these COCs that are obtained during the ovarian tissue cryopreservation. Expansion of the cohort would make it possible to design a prognostic model for clinical use in fertility preservation programs for transgender persons.
Trial registration number: This research is conducted with the approval of the local ethics committee 2015/0124 – B670201523543).

O-094 The difference in size between male and female pronuclei immediately before pronuclear membrane break down (PNMBD) identifies embryos that have potential for successful live birth

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Study question: Can the difference in size between male and female pronuclei (PN) be one of the indicators to tell normal embryos?
Summary answer: Yes, the birth of healthy babies derives from zygotes having similar sized male and female pronuclei, when this measurement is achieved immediately before PNMBD.

What is known already: The size of a male pronucleus is known to be larger than a female pronucleus, however, observation of the size is generally made about 16–20 h after fertilization, although the PN continues to grow until PNMBD.
Study design, size, duration: Retrospective cohort study involving 71 frozen–thawed single blastocyst transfers observed by time lapse system (Embryo Scope) from June 2013 to December 2014.
Participants/materials, setting, methods: Time lapse recordings were performed and the areas of male and female pronuclei were retrospectively analyzed by measuring vertical and horizontal diameter of pronuclei. The measurements were taken 4 h before the PNMBD, which is equivalent to 16–20 h after insemination or ICSI, and right before the PNMBD. The difference in square measurements between the 2PNs in embryos resulting in clinical pregnancy and live born babies were compared to those of embryos from failed pregnancies.
Main results and the role of chance: 71 frozen-thawed blastocysts were transferred after observation with a time-lapse system. The hCG, gestational sack (GC), fetus heart beat (FHB) positive rates were 74.2% (52/71), 63.4% (45/71) and 57.7% (41/71) respectively. Among the 41 cycles with positive FHB, 36 cases were delivered without any chromosomal abnormality, 4 cases miscarried and one case lost contact. The average difference in area (±SD) between 2 pronuclei 4 h before and immediately before PNMBD among patients resulting in the birth of healthy babies were 39.9 μm^2 (±37.8) and 11.6 μm^2 (±15.5) respectively, whereas the average difference in those resulting in unsuccessful birth were 62.8 μm^2 (±43.0) and 62.8 μm^2 (±53.3) respectively. Statistically significant differences were obtained between patients with successful and unsuccessful births both 4 h before ($p = 0.012$) and immediately before ($p < 0.001$) the PNMBD. In addition, the average difference among patients with successful birth was significantly smaller when the measurement was achieved immediately before PNMBD than 4 h before PNMBD ($p < 0.001$). This difference was not detected among patients with unsuccessful birth.
Limitations, reasons for caution: The analysis of PNs is restricted to 2D in this study. 3D analysis will show more precise data when 3D analysis becomes possible. The birth of babies derived from natural conception, which occurs during frozen–thawed HRT cycles, cannot be eliminated in this study, although the occurrence is very small.
Wider implications of the findings: As the size of pronuclei immediately before PNMBD was similar in embryos resulted in the birth of healthy babies, a large difference in size between the 2PN may indicate the presence of aneuploidy, which could be a useful tool in deselecting embryos.
Trial registration number: Not applicable.

O-095 The relation between cell size and developmental stage in human day 2/day 3 embryos is a predictor for blastocyst formation on day 5

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Study question: Does cell size and developmental stage synchronicity in day 2 (D2)/day 3 (D3) embryos has an impact on blastocyst formation on day 5 (D5)?
Summary answer: The percentage of NSS embryos in a cohort of D2 and (or) D3 embryos was 53.5%. Their further developmental potential *in vitro* is reduced.
What is known already: Cleavage-stage embryos that have a correct relation between cell size and embryo developmental stage are called stage specific (SS) embryos while embryos identified with an incorrect relation between cell size and embryo developmental stage are called not stage specific (NSS) embryos. In human preimplantation embryology *in vitro*, little is known about the incidence of SS and NSS embryos and on the developmental consequences of a correct or incorrect relation between cell size and embryo developmental stage.
Study design, size, duration: This study is a retrospective analysis from 25 June 2015 until 31 December 2015. We performed 396 oocyte collection cycles, 4190 oocyte-cumulus cell complexes were retrieved and 3088 MII oocytes were injected by ICSI. After ICSI, 2034 fertilized oocytes (2PN oocytes) were obtained and 1817 were cultured and evaluated until D5 of the oocyte collection cycle (OCC).
Participants/materials, setting, methods: The correlation between cell size and embryo developmental stage was analyzed on D2 and D3. Four embryo categories were identified: Group 1: SS embryos on D2 and D3; group 2: SS embryos on D2 but that developed to NSS embryos on D3; group 3: NSS embryos on D2 but that developed to SS embryos on D3; group 4: NSS embryos on D2 and D3. Statistical analysis was performed by Fisher's exact-test ($p < 0.05$).
Main results and the role of chance: The relation between cell size and developmental stage could be quantified on D2 in 1756 embryos and on D3 in 1629 embryos. On day 2, 1184 (67.4%) embryos were SS embryos and 572 (32.6%) embryos were NSS embryos In the D2 SS embryos 68.8% developed further to SS embryos on D3 (group 1) and 31.2% developed further to NSS embryos (group 2). In the D2 NSS embryos 26.9% of the embryos developed further to SS embryos on D3 (group 3) and 73.1% developed further to NSS embryos (group 4). Interestingly, the distribution of NSS embryos versus SS embryos was significantly different between asynchronously dividing embryos (3-, 5-, 6-, 7-cell embryos) and synchronously dividing embryos (2-, 4- and 8-cell embryos). Significantly more NSS embryos were found in asynchronously dividing embryos as compared to synchronously dividing embryos ($p < 0.05$). Furthermore, In group 1, the blastocyst formation rate was 60.4% (457/757) as compared to 39.5% (136/344) in group 2, 48.6% (69/142) in group 3 and 33.4% (129/386) in group 4 ($p < 0.00001$).
Limitations, reasons for caution: This was a retrospective analysis and the scoring of the relation between cell size and embryo developmental stage was done by several embryologists. The implantation potential of NSS versus SS embryos was not analyzed.
Wider implications of the findings: The correct correlation between cell size and D2/D3 embryo developmental stage is a good predictor for their developmental potential *in vitro*. The findings of this study might contribute in further improving the selection of the embryo with the highest developmental potential.
Trial registration number: NA.

INVITED SESSION

SESSION 26: DO IVF PATIENTS DESERVE STANDARD TREATMENTS?

Tuesday 05 July 2016 **Hall 1** **08:30–09:30**

O-096 Are all patients the same? Adapting protocols methodologies to individual cases

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Since the first successful *In vitro* Fertilization (IVF) treatment was reported almost 4 decades ago, the number of procedures performed every year increase exponentially across the world. The reasons behind this growth are the improvement on its efficiency, and the widening of the type of patients treated. As a consequence, different phenotypes of patients undergo a procedure that in essence is constant in its laboratory aspects, but that can have an equally wide number of different clinical approaches.

Because IVF is a relatively recent medical technique, issues about its adaption to patients' phenotype are controversial. While many other fields of medical practice acknowledge without doubts that individualization of the clinical approach is crucial for the success of the procedure, IVF practitioners still debate if the clinical strategies should be uniform and homogeneous for all types of patients, or should be customized to each patient phenotype.

There is no discussion about the differences among patients undergoing IVF. It is also well established that these differences have a significant impact on the chances of success, such as age, ovarian reserve, body mass index or presence of ovarian dis-functions. However, it is much less clear if considering these features at the time of indicating a particular clinical strategy has a real impact on the probability of success. Very often, the choice between a standardized or an individualized approach, is depending on who ultimately carries with the cost of the procedure. In environments in which the treatment is reimbursed, standardization is the rule, while when the patient pays the procedure out of pocket, the trend is to individualize the treatment.

Those advocating for a standardized practice, claim to inter-cycle variability and to the tremendous influence of Evidence Based Medicine (EBM) to support their position. EBM focuses on the generalization of the results, the

aggregation of data, and the evaluation of the efficacy in average patients. Randomized controlled trials (RCT) have become the keystone for EBM. On the top of that, this type of approach usually involves lower costs per procedure, as less monitorization and time consuming is needed.

On the other hand, defenders of an individualized practice are based on Patient Centered Medicine (PCM). PCM proposes to look back to the individual patient, and tries to provide the best health care to each of them under the conditions of clinical practice, taking into account their preferences as well as their economical resources. While the RCT is the basis for EBM, careful individual observations are the foundation for patient-oriented research.

IVF practice is full of examples of PCM: From the use of different doses and types of gonadotropins to the diverse options of pituitary suppression. Moreover, several co-treatments have been also purposed, according to certain patients' conditions. Unfortunately, most of these strategies are applied in a trial and error basis, far away of what should be proper clinical research. Although some great advances have been done in terms of patients' qualification, especially regarding ovarian reserve evaluation, our field is still lacking of studies on genetic predisposition to treatment response, when compared to others. Furthermore, well-designed RCT in specific subpopulations are scarce, and most of the studies are *post-hoc* sub-analysis. Protocols that have shown to be effective only in ideal patients are often applied to patients with very different characteristics, providing unsurprisingly poorer outcomes.

In summary, despite its clear and crucial benefits, the way to personalized medicine and customized therapy in Reproductive Medicine is still very large, with several barriers to overpass. The integration between clinical research and medical care should define the way to optimize the benefits of IVF for future patients.

O-097 Adopting the same laboratory strategies to all patients. Does it make sense?

J. Hreinsson[1]
[1]*Falun, Sweden*

Assisted reproduction is a technologically oriented field where new methods and advanced treatment modalities are developed continuously. This lecture is intended to be a review of the techniques available to the embryologist today and will offer some guidance and examine evidence for or against various methods to aid in practical decision making in the IVF-laboratory.

Arguments for adopting an individualized approach are that patients vary in terms of age, ovarian reserve, indications for treatment, and quite simply in biological fertility potential. Therefore patients will require various applications of the technological arsenal at our disposal. Simultaneously it is necessary to operate selectively since cost-efficiency, risk/benefit ratios and general minimalist principles dictate that we must not do more than is required to ensure a successful procedure.

To evaluate any method, the underlying biological principles must be taken into account. It is not enough to rely on clinical studies to answer questions regarding new technological developments. Technical difficulties must be overcome, not used as a reason to abandon new methods. This focus on biology leads us to think in a different way regarding the evaluated techniques, focusing on innovation and development: We know that oocytes and embryos are to a large degree susceptible to aneuploidy which may be a reason for miscarriage. It is therefore logical that genetic screening should improve treatment outcome in IVF.

We know that observing a cleaving embryo for less than a minute per culture day gives limited information regarding cleavage progression. Since information on cell divisions will improve embryo selection, then time-lapse imaging should improve success rates.

Knowing that vitrification must occur in all cases of cryopreservation of human embryos, then slow-cooling for 2 h preceding vitrification must have a detrimental effect compared with direct vitrification.

Methods such as assisted hatching, IMSI and chemically induced activation of oocyes will also be discussed and evidence for and against these will be presented.

This way of considering technological advances in assisted reproduction makes it clear that specialization and individualization of treatment modalities must be the best way to further improve our field and ensure optimal treatment for our patients. Simultaneously, it is important to realize that patients may request more than is motivated and professional decision making based on scientific evidence is important to ensure optimal treatment decisions. Competition pressure must not drive clinics beyond professional and academic standards.

INVITED SESSION

SESSION 27: EPIGENETIC REMODELLING IN EMBRYOS: VIEW ON EXPRESSION BY MAGNIFYING GLASS

Tuesday 05 July 2016 **Hall 5 CB** **08:30–09:30**

O-098 Epigenetic landscape in pre-implantation embryo development

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Epigenetic mechanisms are fundamental for the orderly regulation of gene expression throughout the life-course, providing a critical memory of earlier decisions. They can ensure resilience against environmental change, but also allow adaptation. Nowhere is this more important than in the gamete and the early embryo. We are seeking to understand what governs how DNA methylation and other epigenetic marks are patterned during gametogenesis and embryogenesis. Using mouse as a model, we have shown that transcription is the major determinant of the DNA methylation landscape in the oocyte. To understand the mechanistic connection between transcription and *de novo* methylation, we have profiled histone modifications in growing oocytes and identify chromatin states permissive for and resistant to DNA methylation characterised by reciprocal enrichment of H3K4me2/me3 and H3K36me3. This finding is reinforced by the consequences on the DNA methylation landscape of genetically ablating specific H3K4 demethylases and methyltransferases. Key to such studies is the development of methods for genome-wide profiling of epigenetic marks in low numbers of cells, including at the single-cell level, which could have application widely in epigenetic studies, particularly for rare cell types and for investigating cell-to-cell heterogeneity in epigenetic marks at critical developmental transitions.

O-099 Molecular and structural insights into bovine embryonic genome activation

E. Wolf[1]
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Maternal-to-embryonic transition is the period when maternal RNAs and proteins stored in the oocyte are gradually degraded and transcription of the embryonic genome is activated. The onset of embryonic transcription is obscured by the presence of maternal transcripts and could only be determined for genes which are not expressed in oocytes. Using RNA sequencing of bovine germinal vesicle and metaphase II oocytes, and of 4-cell, 8-cell, 16-cell and blastocyst stage embryos, we established the most comprehensive transcriptome data set of bovine oocyte maturation and early development. The embryos investigated were produced by *in vitro* fertilization of German Simmental (*Bos taurus taurus*) oocytes with sperm from a single bull of the genetically distant Brahman (*Bos taurus indicus*) breed to obtain a large number of single nucleotide polymorphisms (SNPs) for identification of the parental origin of transcripts. Further, RNA-Seq libraries were produced without polyA+ selection enabling the identification of intronic sequences in transcripts, which can be found in de novo synthetized transcripts due to incomplete co-transcriptional splicing and can thus be used to discriminate them from spliced maternal transcripts stored in the oocyte.

In all developmental stages investigated, transcripts from 12.4 to 13.7×10^3 different genes were detected. Our differential gene expression analyses revealed only few transcripts to be differentially abundant between the GV and MII oocyte and between MII oocyte and four-cell stage. According to the main embryonic genome activation we found a marked increase in the number of differentially expressed genes between four- and eight-cell stage and even more between the later stages. The proportion of transcripts with decreased abundance gradually increased during embryonic development from 17% between four-cell embryo and MII oocyte to about 55% between 8- and 16-cell embryos, which could be due to the degradation of maternal transcripts.

EGA was analyzed by (i) detection of embryonic transcripts which are not present in oocytes; (ii) detection of transcripts from the paternal allele; and (iii) detection of primary transcripts with intronic sequences. Using these approaches we were able to map the onset of embryonic transcription for almost 7,400 genes. A functional classification of the 414 genes found to be activated in bovine embryos at the four-cell stage or before revealed the gene ontology (GO) terms "RNA processing," "Translation" and "Transport," indicating the first transcriptional activity before major EGA. Our RNA-Seq study was able to detect the onset of embryonic expression for 4,489 specific genes at the eight-cell stage. GO classification of the eight-cell activated genes revealed the functional categories "Transcription, DNA-dependent," "Purine nucleotide metabolic process," "Protein ubiquitination," "Translational initiation" and most prominently "RNA metabolic process" to be significantly overrepresented. In addition, the GO classification of genes activated in later developmental stages revealed new roles and functions as compared to the earlier stages.

In summary, RNA-Seq analyses provided new insights into embryonic genome activation, allelic expression of genes, and transcriptional networks governing early development of embryos. RNA-Seq data provide a fundamental basis for embryo biosystems research, including germ cell potential and genetic, epigenetic and environmental disturbances of early development. In addition, studies based on structured illumination microscopy revealed major changes in higher order chromatin organization associated with major EGA.

INVITED SESSION

SESSION 28: ASRM EXCHANGE SESSION - SPECIAL CONSIDERATIONS FOR ART IN PATIENTS WITH SEX CHROMOSOME ANEUPLOIDY: KLINEFELTER SYNDROME AND TURNER SYNDROME

Tuesday 05 July 2016 **Hall 5 A** **08:30–09:30**

O-100 Klinefelter syndrome

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Klinefelter Syndrome (KS) is the most common chromosomal disorder in men, and the most common cause of primary testicular failure and azoospermia. Prevalence is estimated to be between 1:500 and 1:1000. However the actual prevalence is probably greater owing to the historically frequent failure to identify and diagnose these patients. The expansion of noninvasive prenatal testing should lead to an increase in the number of diagnosed KS patients. In addition to the classic phenotype of the patient with seminiferous tubule dysgenesis and androgen deficiency, the spectrum of KS includes a number of equally important medical disorders: motor, cognitive and behavioral dysfunction; tumors; vascular disease; and endocrine, metabolic, and autoimmune diseases. Earlier diagnosis of KS may lead to earlier treatment of all aspects of the syndrome, including possible fertility optimization and preservation. The testicular function of KS patients progressively declines with age. Prepubertal KS boys have levels of testosterone comparable to non-KS boys until puberty when T levels begin to rise, only in turn to decrease by age 15 years in the majority of KS boys. Similarly, testicular histology is mostly normal in infancy, with progressive loss of spermatogonial stem cells at the onset of puberty, with progressive fibrosis and hyalinization of the seminiferous tubules and Leydig cell hyperplasia by adulthood. Because few KS men have sperm present in the semen, these men were considered incapable of fathering a child until ICSI and ART revolutionized their treatment. Success rates of sperm recovery by TESE in adult XXY men have been reported to be between 20 and 70%, with live birth rates of up to 50%. Genetic counseling of the KS patient in the ART setting is essential. Recently, because of the potential for earlier diagnosis of KS, focus has shifted to timing of initiation of testosterone therapy, cryopreservation of semen samples from KS boys in early puberty, pre-pubertal testicular biopsy and cryopreservation, and hope for subsequent *in vitro* maturation for use in future ART procedures. The latter proposed approaches are still in the research setting and require further validation and careful attention to ethical considerations.

O-101 Turner syndrome

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Abstract text

Turner syndrome (TS) occurs at a rate of 1 in 2,500 female infants. A 45,X karyotype is very lethal: 99% of concepti are estimated not to survive first and second trimester and 15% of spontaneous abortions are associated with this monosomy. Historically, TS was the most common cause of delayed female puberty and found even as nonmosaic 45,X in women with adult onset ovarian insufficiency. Karyotypic-phenotypic relationships are not as easily made as clinically thought but recent findings suggest that those individuals identified prenatally have fewer findings than those ascertained in childhood or adolescence. In addition to pheontypic findings, this lecture will discuss the known genetic and ovarian etiologies for follicular and oocyte loss. It will present data on when that loss occurs as well as the proportion of women with Turner syndrome who have degrees of ovarian function before the premature insufficiency. While it was previously estimated that 5% of TS women will have menstrual function, 1% may become pregnant. Data regarding prior pregnancies and those requiring IVF with their own oocytes are presented. In addition, the option of freezing oocytes in young TS women who are still menstruating is discussed. For many years, however, the non Obstetrics and Gynecology literature reported the occurrence of aortic dissection and rupture for TS women in the nonpregnant state; an occurrence 500–2,000 times more frequent than for the general population and at a usual age between 20 and 40 years rather than the average age of 63 for the general population for which men outnumber women. Risk factors for aortic dilation, dissection and rupture are presented including congenital heart disease (especially bicuspid aortic valves and coarctation), known dilation and hypertension. TS women have the same histopathology of their vessels, cystic medial necrosis, as do those patients with Marfan syndrome. With a know predisposition for aortic dissection and rupture in the nonpregnant state, it was predicted that the increased blood volume, pulse pressure, and stroke volume would increase this risk during pregnancy. The data demonstrating this reality are presented. In addition to increasing the risk of death during pregnancy by an estimated 100-fold over all cause mortality, data are accumulating that overall morbidity is also increased, especially for hypertensive disorders of pregnancy. The question looming is whether the risk of maternal mortality is sufficiently high such that TS is an absolute contraindication to pregnancy? Further complicating this important question is the lack of registry data to understand the true prevalence of this occurrence and the fact that there has yet been a TS phenotype for which death has not occurred. For example, TS patients without overt risk factors have had dissection and rupture. In addition, TS women who have had spontaneous menses up through their 30s have had rupture as have those with a prior spontaneous pregnancy. A call to action for collecting these data by registry and potential future surgical options are presented.

INVITED SESSION

SESSION 29: PARAMEDICAL INVITED SESSION - NURSING

Tuesday 05 July 2016 **Room 101** **08:30–09:30**

O-102 Fertility preservation for transgender prior to transition surgery

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[2]*University Hospital Ghent, Department of Endocrinology and Center for Sexology and Gender Problems, Ghent, Belgium*

Abstract text

Hormonal and surgical treatments for transgender people have a devastating effect on the possibility for these patients to reproduce. Additionally, transgender people tend to start sex reassignment treatment at a young age, when reproductive wishes are not yet clearly defined nor fulfilled. The most recent Standards of Care of the World Professional Association for Transgender Health recommend clearly informing patients regarding their future reproductive options prior to initiation of treatment. Transgender patients are therefore a growing patient population visiting our fertility centers for advise on how to fulfill their future child wish or seeking fertility preservation treatments. Where genital reconstructive surgery definitely results in sterility, hormone therapy on the other hand also has an important, but partially reversible impact on fertility. The current fertility preservation options for trans men are embryo cryopreservation, oocyte cryopreservation and ovarian tissue cryopreservation. For trans women, sperm cryopreservation, surgical sperm extraction and testicular tissue cryopreservation are possible. Although certain fertility preservation techniques could be applicable in a standardized manner based on clear biological criteria, the technique that eventually will be performed should be the preferred choice of the patient after extended explanation of all possible options. I will discuss the possible options that are available for transgender people now or may be possible in the future based on a recent review of our group by De Roo et al. (2016) (*Int. Rev. Psychiatry*). Although, theoretically, both trans women and trans men are capable of using fertility preservation treatments, there is still an important psychological burden for trans women to masturbate in order to produce sperm, of for trans men to undergo repeated endovaginal ultrasound monitoring to eventually collect oocytes for subsequent vitrification. At Ghent University Hospital, the use of *in vitro* maturation of oocytes collected at the time of transition surgery upon cryopreservation of ovarian tissue is being explored as a future promising realistic fertility preservation technique for trans men. Preservation and freezing of ovarian tissue is often chosen as fertility preservation technique by young trans man: no additional controlled ovarian stimulation by vaginal ultrasound is needed and oophorectomy is standard, at least in Belgium, during sex reassignment surgery. During the manipulation of the ovarian tissue, cumulus-oocyte complexes can be collected and *in vitro* matured up to the MII stage. These oocytes display a normal spindle structure and chromosome alignment showing that supraphysiological doses of testosterone do not have an effect on recovered and *in vitro* matured oocytes in trans men. Do these oocytes exhibit normal fertilization and embryo development is a question we are in the process of answering. If this would be the case, this technique could definitely maximize fertility preservation option for trans men and give them a real option for fulfilling their future child wish with their own gametes. In summary, with this presentation I will discuss the theoretical part on fertility preservation in transgender people, share with you our experiences with this specific group of patients and show recent results from our research in transgender fertility preservation.

O-103 Are nurses and midwives competent enough to perform ultrasound monitoring in ovarian stimulation?

J. Schoonenberg-Pomper[1]
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Abstract text

Over the past decades the number of clinical tasks in fertility clinics, which are performed by nurses or midwifes have increased. In fertility care one of the tasks, which nurses and midwifes are starting to perform, is ultrasound monitoring during ovarian stimulation.

One could question whether nurses are competent enough to perform this task. In 2009, the ASRM wrote a position paper supporting nurses to perform ultrasound monitoring during ovarian stimulation. This paper pleads that nurses performing ultrasound monitoring need a bachelor degree, theoretical training and training on the job. Furthermore, the clinics allowing the nurses to perform this task need specific guidelines on ultrasound monitoring and need make sure that a physician is available for supervision, if needed, while the nurses perform their scans. A recent study did show that midwifes have a high inter- and intra-reliability while performing ultrasound scanning during ovarian stimulation.

One could question whether asking nurses or midwifes to perform ultrasound monitoring during ovarian stimulation will affect the safety and effectiveness of fertility care. There is no study that compares the pregnancy rates and risks of fertility treatments depending on whether a physicians or a nurse/midwife performs the ultrasound monitoring during ovarian stimulation. Nevertheless, clinics in which nurse and/or midwifes have started to perform the ultrasounds monitoring do not see a decline in pregnancy rates. Research from other medical fields showed that effectiveness and safety outcomes are not affected by nurses/midwifes performing an ultrasound (e.g., ultrasound scanning during a peripheral intravenous (IV) catheter placement). Professionals often focus on effectiveness or more specifically pregnancy rates or live birth rates or safety when talking about improving the quality of fertility care. However, one in three compliant couples will not go home with a baby.

One could question whether asking nurses or midwifes to perform ultrasound monitoring during ovarian stimulation will affect other dimension of quality of fertility care than its safety and effectiveness. According to the "Institute of Medicine" quality of care depends on timeliness, efficiency, equity, and patient-centeredness besides effectiveness and safety. Regarding timeliness and efficiency, allowing nurses and midwifes to perform more clinical tasks can resolve the documented waiting lists for treatment in clinics, in which physicians are understaffed.

In addition, research from other medical fields indicated that giving more clinical tasks to nurses or midwifes can improve the patients-centeredness of care. Research showed that patients do not only want the highest chance of pregnancy but they want their care to be patient-centered. The patient-centeredness of care is defined by ten dimensions; provision of information, competence of clinic and staff, coordination and integration, accessibility, continuity and transition, physical comfort, attitude of and relationship with staff, communication, patient involvement and privacy and emotional support. The fact that allocating more tasks to nurses or midwifes can improve patient satisfaction with patient-centered care can be explained by the attention given to communication, education and emotional support in nursing and midwifery education and by the fact that nurses and midwifes take out more time for their patients.

Finally, research from other medical fields also showed that nurse and midwifes are more willingly to work within the boundaries of guideline, which might in turn result in improving the quality of care.

In conclusion, trained nurses and midwife can perform ultrasound monitoring during ovarian stimulation. It seems that this could improve several dimension of quality of care while maintaining pregnancy rates. This should, however, be confirmed by high quality randomized controlled trials.

SELECTED ORAL COMMUNICATIONS

SESSION 30: IVF LABORATORY QUALITY AND STRATEGIES

Tuesday 05 July 2016 **Hall 1** **10:00–11:30**

O-104 Evaluation of the effects produced by volatile organic compounds (VOCs) over human embryo development

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Study question: Can specific concentrations of common IVF ambient air VOCs produce adverse effects over the human embryo *in vitro* development?

Summary answer: Embryos cultured along with IVF ambient air- related concentrations of VOCs, such as benzene and limonene, have worst embryo score and reduced embryo survival rates.

What is known already: Previous studies have found a correlation within the presence of harmful VOCs and adverse effects on fertility (low implantation or pregnancy rates); however, there are few studies correlating the presence of VOCs inside the IVF and specific adverse effects over *in vitro* embryo development. At the same time, facilities manage occupational limit values (OLV) inside the IVF laboratories to daily cover exposed workers without adverse health effects, but specific quality standards and specific threshold levels, at which most of the common pollutants in the IVF`s ambient air can cause harm to cultured human embryos, have not been determined.

Study design, size, duration: Prospective cross sectional study with 400 human embryos, to perform a comparative analysis within 25 control (C) embryos and groups of 75 embryos randomly cultured from day 3 (D3) up to

day 6 (D6) of development, and exposed to 5 VOCs (recognized as harmful ambient air pollutants). Each compound has 3 experimental groups (EG), related to the atmospheric VOCs concentrations determined in routinely IVF`s environmental characterizations: exterior (E), laboratory (L) and double laboratory (D).

Participants/materials, setting, methods: Each developmental toxicity test (DT) exposes D3 embryos, with 6- 10 cells and less of 25% of fragmentation, by contaminating CCM™ blastocyst culture medium with 3 doses of Benzene, Limonene, Styrene, 1,1,2,2-Tetrachloroethane and Hexachloro-1,3-butadiene; doses are under 1% of the OLV. After thawing, 25 embryos are exposed to each dose up to D6 of development and embryo grading is performed. Biopsy of trophectoderm (TE) is done for future chromosomic and methylation analysis.

Main results and the role of chance: Up to now, 102 out of the 400 embryos have been thawed and cultured; 93 have been exposed to VOCs. First DT: we exposed 75 embryos to Benzene (B): BE = 268×10^{-5} ppm, BL = 256×10^{-5} ppm, BD = 512×10^{-5} ppm. 62 out of 75 (82.7%) developed to blastocyst stage, of which 77.4% ($n = 48$) reached expanded to hatched status. Inner cell mass (ICM) and TE good quality scores (A-A, A-B, B-A, B-B) were only registered on 23/48 developed embryos (47.9%). Morphologically there were A-A score embryos only in dose groups BE and BD ($n = 4$ in each); however other good scores were registered in all groups: BE = 6, BL = 2, and BD = 6. Second DT: so far, we have exposed 18/75 embryos to Limonene (L): LE = 87.5×10^{-5} ppm, LL = 113.6×10^{-5} ppm, LD = 226×10^{-5} ppm. 17 out of the 18 (94.4%) reached blastocyst stage; 72.2% ($n = 13$) reached expanded or initiating hatching status. ICM and TE good quality have been seen in 7/17 embryos (41.2%). A-A scores have not been observed but other good scores were seen in the three groups: LE = 3, LL = 3, LD = 1. In the control group ($n = 9$), 100% of embryos reached different blastocyst stages; 55.5% ($n = 5$) had good scores but none of them have scored A-A either.

Limitations, reasons for caution: Results are being obtained by direct contamination of the culture medium with VOCs, which does not represent exact laboratory conditions. Relationship of blastocyst stage with early scores will be analyzed to follow individual pattern of development, and ploidy status should be obtained as well, to correlate with the morphology.

Wider implications of the findings: Associating specific morphologic, chromosomal and molecular results, produced over the embryos by the most common ambient air VOCs inside the IVF, an official database can be established, along with health regulatory institutions, with accurate OLVs for human embryo culture.

Trial registration number: N/A.

O-105 Worse by the hour: *in vitro* aging of oocytes lowers the chance of achieving a pregnancy but does not affect live birth rates.

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Study question: Does the aging of *in vitro* of oocytes between ovum pick up (OPU) and ICSI affect reproductive outcomes after fresh embryo transfer?

Summary answer: Biochemical and clinical pregnancy rates are lowered by *in vitro* oocyte ageing, however, live birth rates after fresh embryo transfer (ET) are not.

What is known already: Appropriate oocyte cytoplasmatic and nuclear maturation are of paramount importance in order to ensure an optimal embryonic developmental competence. While nuclear maturation is usually attained by the time an oocyte reaches OPU, cytoplasmic maturation cannot be readily assessed and might be incomplete. On the other hand, excessive *in vitro* aging of human oocytes affects their ultrastructural characteristics and, in mice, induces alterations in expression and epigenetic modifications of chromatin and histone patterns. Defining an optimal moment to inseminate after OPU might have a positive effect on embryo development and reproductive outcomes.

Study design, size, duration: Retrospective study including 1,478 ICSI cycles between December 2012 and September 2015. All ET were fresh. *In vitro* ageing times studied: OPU–denudation (DN); DN-ICSI and OPU-ICSI. A radiofrequency based system was used to record exact times. The effect of total and partial time intervals between procedures, from OPU to ICSI, on fertilization rate and on biochemical, clinical, ongoing pregnancy and live birth rates were analyzed.

Participants/materials, setting, methods: Differences in biochemical, clinical, ongoing, and live birth rates were tested by Mann–Whitney *U* test. The likelihood of positive clinical outcomes was further modeled by logistic regression, adjusting for woman's age and body mass index (BMI), day of ET, number of transferred embryos and mean embryo quality, sperm origin (partner/donor) and status (fresh/frozen), and number of mature oocytes obtained at OPU. Effect of time on fertilization rate was modeled by probit regression.

Main results and the role of chance: The mean woman age was 38.4 (SD 4.6). Overall biochemical, clinical, ongoing pregnancy rates, and live birth rate were: 38.3, 29.8, 21.7 and 20.0%, respectively. Mean times in hours for OPU-DN, DN-ICSI and OPU-ICSI were: 1.0 ± 0.4, 4.7 ± 2.9 and 5.7 ± 2.9, respectively, and were not different for pregnant and non pregnant patients. However, the multivariate analyses showed that on average (anti-log transformed), each 60-min increase in the OR-ICSI time reduced the likelihood of biochemical pregnancy by 7.8% (95%CI 1.2–14.9%) and of clinical pregnancy by 7.9% (95%CI 1.1–15.2%). No effect of time was observed for ongoing pregnancy or live birth. Similar effects were observed for the DN-ICSI times (OR 0.74, 96%CI 0.59, 0.92, $p = 0.008$ for biochemical and OR 0.77, 96%CI 0.61, 0.98, $p = 0.032$ for clinical pregnancy), whereas OPU-DN time did not show to have a significant effect on PR. Increasing OPU-ICSI time reduced the fertilization rate ($B = 0.28$, $p < 0.01$). Again, this effect was mainly due to an increase in DN-ICSI time while OPU-DN had no statistically significant effect.

Limitations, reasons for caution: The main limitation of this study is its retrospective nature. The lack of relationship between *in vitro* ageing and live birth rates might be due to uncontrolled variables. These results should not be extended to other ART protocols such as *in vitro* maturation of oocytes or classical IVF fertilization.

Wider implications of the findings: This study indicates that *in vitro* ageing of oocytes significantly affects the chance to become pregnant. Effect on live birth rates, although not evident in this study, cannot be excluded. Limiting *in vitro* ageing in the IVF lab might improve reproductive results for most patients.

Trial registration number: NA.

O-106 The role of intracytoplasmic sperm injection (ICSI) for non-male factor infertility in women aged 40 and over

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Study question: Does intracytoplasmic sperm injection (ICSI) improve pregnancy and live birth rates when compared to conventional *in vitro* fertilization (IVF) in women aged 40 and over?

Summary answer: There is no improvement in pregnancy and live birth rates with ICSI vs. conventional IVF in women aged 40 and over with non-male factor infertility.

What is known already: ICSI is an effective treatment for male factor infertility but it is increasingly being used for patients without male factor, too frequently without evidence-supported indications. The effectiveness of this technique has not been adequately studied outside of male factor infertility. In the 2012 American Society for Reproductive Medicine committee opinion regarding the use of ICSI for non-male factor infertility, it is stated that there are no studies looking at the benefit of ICSI in advanced maternal age. This is the first study to investigate the value of ICSI when normal semen parameters are present per the 2010 WHO criteria.

Study design, size, duration: This retrospective cohort study involved 720 women, aged 40–43 years, who underwent IVF between January 2012 and December 2014 for non-male factor infertility. 229 had conventional IVF, 491 used ICSI for fertilization. Women <50% fertilization at previous IVF were excluded. Patients were included once. All eligible subjects were included. Subjects had a semen analysis with at least: 1.5 ml, 15 million sperm/ml, 39 million total sperm, progressive motility 32%, total motility 40% and morphology 4%.

Participants/materials, setting, methods: All subjects had at least 1 year of primary or secondary infertility. Participants were treated with either the midluteal long gonadotropin releasing hormone agonist (GnRH-ag), GnRH-ag microdose flare or GnRH antagonist protocols. Indications for care included tubal factor, advanced age, unexplained infertility, decreased ovarian reserve,

and anovulation having failed ovulation induction with insemination. Statistical analyses were performed using logistic regression to control for confounding effects. Data is presented as xÌ ± SD or as percentages.

Main results and the role of chance: Patient characteristics were similar between the two groups with respect to maternal age (p = 0.736), paternal age (p = 0.159), total number of oocytes collected (p = 0.120), BMI (p = 0.207), FSH dose (p = 0.119), protocol type (p = 0.188) and number of smokers (p = 0.232). The only differences between the groups noted were number of MII oocytes collected (ICSI 5.1 ± 3.82, IVF 6.1 ± 4.55; p = 0.002), day of transfer (ICSI 3.3 ± 1.2, IVF 3.5 ± 1.2; p = 0.003) and previous IVF attempts (ICSI 1.1 ± 1.0, IVF 0.65 ± 0.9, p = 0.001). Rates of fertilization failure did not differ between ICSI and IVF groups (3 vs. 4% respectively, p = 0.57). IVF resulted in more blastocyst formation than ICSI (p = 0.008). Pregnancy rates were comparable (ICSI 24.6%, IVF 29.7%; p = 0.449) as were live birth rates (ICSI 10.2%, IVF 11.9%; p = 0.345) when controlling for confounders.

When sub-selecting patients with decreased ovarian reserve (defined as total FSH dose ≥3,000 IU or ≤3 MII oocytes retrieved), clinical pregnancy and live birth rates did not differ between IVF and ICSI (FSH dose ≥3000 IU (N = 506): p = 0.78, p = 0.58 respectively) (MII 3 (N = 99): p = 0.45, p = 0.80 respectively).

Limitations, reasons for caution: This retrospective cohort study was not randomized therefore this may be a source of undetected bias. However, given the robust sample size, it is unlikely that these results are due to chance alone and the probability of bias is minimized.

Wider implications of the findings: ICSI is often used in women of advanced age to prevent unforeseen failed or low fertilization. This study suggests ICSI does not improve pregnancy outcomes or fertilization rates. Conventional IVF improves blastocyst formation rate, which may offer benefit. ICSI in women aged 40–43 with normal semen parameters is not supported.

Trial registration number: N/A.

O-107 Pre-zygotic transfer (PZT): a pilot study in poor prognosis patients

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Study question: Can transfer of one pre-zygote (PZ) to the uterus within 60 min of extruding the second PB result in live births for poor prognosis patients?

Summary answer: 5 live births and 0 miscarriages from 69 PZTs compared with 4 live births and 7 miscarriages from 191 previous embryo transfers.

What is known already: Despite improved embryo selection using extended culture and time-lapse recording, there remains a group of poor prognosis IVF patients for whom live births are rare. In rat studies, the environment during pronuclear (PN) formation was critical for live births, and significantly lower birth rates arose from oocytes fertilized *in vitro* compared with oocytes fertilized *in vivo*. Extrusion of the second polar body 2–3 h after ICSI is predictive of normal fertilization, and transfer of PZs after extrusion of the second PB should eliminate any risk of transferring abnormally fertilized oocytes and may improve outcomes for poor prognosis patients.

Study design, size, duration: A prospective study of 43 women with previous poor cycle outcomes and/or advanced age was conducted in Mio Fertility Clinic from August 2012 to August 2015. Participants had an average age of 38.5 years (31–46) and they had 203 (range 1–13) previous IVF/ICSI cycles and 191 single embryo transfers (sET) resulting in only 4 term pregnancies and 7 miscarriages. All participants gave informed consent for the trial.

Participants/materials, setting, methods: Oocytes were collected in stimulated cycles and cultured for 2–6 h. Mature oocytes were injected with one sperm and then cultured in a time-lapse imaging incubator (Embryoscope®), so extrusion of the second polar body could be clearly identified and timed. Single PZs with a second polar body were transferred to the uterus under ultrasound guidance within 60 min of the second polar body being extruded.

Main results and the role of chance: 43 women completed 69 PZT cycles. A total of 574 oocytes were collected in these cycles, of which 419 had extruded the first polar body. ICSI resulted in 69 PZs that were transferred within 60 min of second polar body extrusion, and 218 normally fertilized (2PN, 2PB) oocytes that were cultured for cryopreservation. In the 69 PZs, the second PB was extruded 2.71 ± 0.73 h post-ICSI. A similar pregnancy rate between PZT and routine sETs (7.2% cf 5.4%, p = 0.66) was observed. However, significantly better outcomes, five live births (7.2%), were established compared to four live births (1.9%) in the 191 previous embryo transfers (p = 0.04).

Limitations, reasons for caution: The utility of PZT has not been established in good prognosis patients, and the low number of pregnancies means the Central Limit Theorem may not apply and the resultant p values may be invalid.

Wider implications of the findings: These PZT results demonstrate that the uterus can support the development and implantation of pre-zygotes, and *in vivo* development may improve pregnancy outcomes for poor prognosis patients. Furthermore, PZT may provide a viable low cost alternative to extended culture, especially if combined with minimal stimulation without cryopreservation.

Trial registration number: None.

O-108 Cleavage-stage embryo cryopreservation/warming and blastocyst stage embryo transfer is more beneficial over blastocyst stage embryo cryopreservation/warming strategy in good responder patients

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[4]*Bahceci Umut IVF Centre, Infertility Clinic, Istanbul, Turkey*

Study question: This study is performed to evaluate the possible beneficial effects of cleavage- and blastocyst-stage embryo cryopreservation, subsequent warming/culture and transfer at the blastocyst stage strategies.

Summary answer: Cleavage-stage embryo cryopreservation, culture after warming and subsequent transfer at blastocyst stage is more beneficial over blastocyst-stage embryo cryopreservation and warming.

What is known already: High survival and implantation rates after transfer of vitrified embryos lead many IVF clinics to adapt to freeze-all policy progressively. There exists a tendency to cryopreserve embryos at the blastocyst-stage, especially in good responder patients. Several studies have already shown acceptable results with frozen embryo transfer (FET) cycles in comparison with fresh embryo transfers. However, the number of studies analyzing the efficiency of FET over fresh ET at various embryo developmental stages is scarce and there is a lack of consensus over which stage of embryo cryopreservation is optimal for optimal clinical outcome.

Study design, size, duration: This retrospective cohort study includes 1,497 antagonist cycles performed between January 2013 and December 2015 in Bahceci Fulya IVF Centre. Good responder female patients with ≤38 years of age and male partners with normo-oligospermia were enrolled into the study. Exclusion criteria included poor response to controlled ovarian hyperstimulation, repeated implantation failure (≥3 IVF failure), pooling embryos (more than 2 oocyte pick-up cycles without embryo transfer) and using testis sperm for ICSI.

Participants/materials, setting, methods: Cycles included in the study were grouped into three: Group I included cycles in which embryos were cryopreserved & warmed on day 3, cultured to day 5 and transferred; Group II consisted of cycles in which embryos were cryopreserved & warmed on day 5/transferred on day 5; and Group III included cycles in which embryos were transferred on day 5 as fresh, without any prior cryopreservation. Every patient involved in the study only once.

Main results and the role of chance: The differences in clinical outcome among three study groups were compared. Patient characteristics such as age, BMI and sperm parameters were comparable among groups (p > 0.05). There were statistical significant differences between biochemical (BPR) and clinical pregnancy rates (CPR) among groups (p < 0.001). CPR in Group I (75.3%) was significantly higher than both Group II (65.2%) and Group III (63.0%) respectively (p < 0.001 p = 0.005). On the other hand, similar pregnancy rates were observed between Group II and Group III (p = 0.602). There were also no statistical significant differences among abortion rates in all groups (18.5, 13.5 and 15.9% respectively; p = 0.198).

Limitations, reasons for caution: The main limitation of our study was its retrospective nature, since numerous confounding factors that can affect the ART outcome may be overlooked. Also, cumulative pregnancy rates of cycles included in the study have not been taken into account in this analysis.

Wider implications of the findings: Our study indicates that extended embryo culture after warming may positively affect the pregnancy outcome. However,

well-designed additional prospective studies with large sample sizes will be needed to draw a firm conclusion on which development stage embryos must be cryopreserved.
Trial registration number: None.

O-109 Development of a normogram for early warning based on the relationship of key performance indicators with fresh *in vitro* fertilization cycle outcomes

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Study question: Can deterioration of key performance indicators (KPI) serve as early warning signs (EWS) for early intervention and improvement in the *in vitro* fertilization (IVF) laboratory?
Summary answer: Higher clinical pregnancy rates (CPR) were significantly associated with KPI remaining above the mean and lower CPR with clustering of KPI below the mean.
What is known already: KPI which include metaphase-II oocyte (M-II), fertilization, cleavage and embryo quality rates depend upon oocyte quality and IVF laboratory conditions, e.g., temperature, pH, humidity, air quality, disposables and media etc. The impact of deterioration in IVF laboratory quality is reflected in CPR and implantation rates (IR). However, these indices are available much later. There is no way to go back in time and institute remedial interventions in order to salvage the remaining cases. Moreover it is very difficult to evaluate the offending factors retrospectively. EWS, if available can give timely alerts and help in trouble shooting.
Study design, size, duration: This is a retrospective study of 259 fresh IVF embryo transfer (ET) cycles over a period of 1 year. KPI analysed were M-II, fertilization, cleavage and embryo quality rates. Comparison was done using independent *t*-test between KPI of β human chorionic gonadotrophins (β-hCG) and clinical pregnancy (CP) positive and negative groups. Normograms were charted using mean of KPI with error bars set at ±3 standard deviations (SD) and tested against CPR and IR.
Participants/materials, setting, methods: The cases included were standard IVF cycles and fresh ET conducted at a tertiary care center in northern India. β-hCG positivity was considered with blood level > 100 mIU/ml on day-14 post ET and clinical pregnancy positivity was diagnosed with minimum one gestational sac with cardiac pulse detected on ultrasonography done after 6 weeks post ET. All cases were performed in 20 batches, each lasting for 12–15 days.
Main results and the role of chance: In β hCG positive group, the mean M-II, IVF fertilization, ICSI fertilization, cleavage and embryo quality rates were 87.98, 93.19, 94.12, 91.97 and 93.02% respectively while in pregnancy negative group, these were 80.37, 88.96, 86.14, 86.21 and 89.71% respectively. In CP positive group, the mean M-II, IVF fertilization, ICSI fertilization, cleavage and embryo quality rates were 88.21, 93.01, 95.43, 93.00 and 93.83% respectively while in pregnancy negative group, these were 81.47, 89.57, 86.31, 86.72 and 89.78% respectively. These differences were significant for M-II, ICSI fertilization and cleavage rates (p = 0.021, 0.001 and 0.001 respectively) and insignificant for IVF fertilization and embryo quality rates (p value = 0.269, 0.060 respectively). The highest positive correlation of β hCG positivity, CPR and IR was observed with cleavage rate (standardized β coefficient = 0.422) amongst various KPI analysed. During the batch with worst outcome, a decline in KPI of more than one standard deviation from the mean was observed. A fall in KPI of more than two standard deviations from mean and clustering of events over period of 1–2 days was associated with the poor outcome. IVF outcome was better when KPI remained above the mean values.
Limitations, reasons for caution: Besides KPI, there are many other variables which may affect the IVF outcome like oocyte quality, endometrium, day and number of embryos transferred. These factors were not considered in analysis. A knee jerk reaction and changes in IVF laboratory protocol on the basis of few events may also be counterproductive.
Wider implications of the findings: The normograms of KPI of individual IVF laboratory can serve as objective markers for evaluation of any intervention in IVF laboratory protocols. A decline and clustering of daily events charted over these normograms should serve as warning signs.
Trial registration number: Since it was a retrospective analysis of data, trial registration number was not sought.

INVITED SESSION

SESSION 31: LIVE SURGERY SESSION

Tuesday 05 July 2016 **Hall 5 CB** **10:00–13:00**

O-110 The added value of reproductive surgery

S. Gordts[1]
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SELECTED ORAL COMMUNICATIONS

SESSION 32: ENDOMETRIAL RECEPTIVITY - WHAT'S NEW?

Tuesday 05 July 2016 **Hall 5 A** **10:00–11:30**

O-111 Why do we still measure endometrial thickness and what is its effect on live birth rates?

S. Santos-Ribeiro[1,2], V. Costa Ribeiro[3], N. De Munck[1], P. Drakopoulos[1], N. P. Polyzos[1,4], V. Schutyser[1], G. Verheyen[1], H. Tournaye[1], C. Blockeel[1,5]
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[2]*Santa Maria University Hospital, Department of Obstetrics Gynaecology and Reproductive Medicine, Lisboa, Portugal*
[3]*Centro Hospitalar Lisboa Central, Maternidade Dr Alfredo da Costa, Lisboa, Portugal*
[4]*Faculty of Health University of Aarhus, Department of Clinical Medicine, Aarhus, Denmark*
[5]*School of Medicine of the University of Zagreb, Department of Obstetrics and Gynaecology, Zagreb, Croatia*

Study question: Is there any added-value in assessing endometrial thickness (EMT) on the day of ovulation triggering to predict (LBR) in modern-day *in vitro* fertilization (IVF)?
Summary answer: An increase in EMT is independently associated with an increase in LBR.
What is known already: Previous studies have shown that EMT has a controversial effect on IVF pregnancy outcomes. Specifically, while some researchers have shown that it may affect surrogate outcomes, such as clinical pregnancy and miscarriage rates, studies thus far have failed to show any influence on live birth. However, the extrapolation of these results to modern-day practice may be limited by their sample size and potential unmeasured confounding factors, such as body mass index (BMI) and the late-follicular-phase endocrine profile. With the constant enhancement of IVF, one can only postulate if EMT still has any role in predicting pregnancy outcomes.
Study design, size, duration: We performed a single-centre retrospective cohort analysis of 3,351 gonadotropin-releasing hormone (GnRH) antagonist down-regulated cycles with a fresh embryo transfer performed in a tertiary hospital between January 2010 and October 2014. The sample was categorized according to the following regular 2-millimeter-intervalled groups of EMT on the day of ovulation triggering: <7.0 mm, 7.0–8.9 mm, 9.0–10.9 mm, 11.0–12.9 mm and 13 mm. LBR was the main outcome measure.
Participants/materials, setting, methods: Cycles using preimplantation genetic diagnosis, *in vitro* maturation and donor oocytes were excluded. We performed multivariable regression adjusting the standard errors for the clustering of cycles repeated in the same patient and accounting for the following potential confounders: female age, BMI, cycle rank, total dose of exogenous follicle-stimulating hormone (FSH), the late-follicular-phase endocrine profile [specifically, estradiol (E2) and progesterone (P) levels], the number of embryos produced and the number, stage and grade of the embryos transferred.
Main results and the role of chance: The mean female age of our sample was 32.5 years and 62.3% of the patients were undergoing their first or second IVF attempt.

An increase in BMI was linearly related to an increase in EMT. On the other hand, the following variables were associated with a significant decrease in EMT on the day of ovulation triggering: total FSH dose <750 IU, late-follicular

E2<1,000 pg/mL or P < 0.5 ng/mL. All variables with an effect on EMT were considered as potential effect-modifiers of the influence of EMT on live birth and assessed for their significance as interaction terms in the multivariable logistic regression model.

In the multivariable logistic regression model, EMT was an independent predictor of live birth in our sample, even after accounting for the before-mentioned potential confounders (predicted live-birth rates by ascending order of EMT thickness: 18.2, 25.4, 28.7, 30.2 and 26.2%, respectively).

Limitations, reasons for caution: This study should be interpreted with caution owning to its retrospective design and the potential for unmeasured confounding such as female smoking habits and previous uterine surgery. Furthermore, these results should not be extrapolated to other perinatal outcomes, such as prematurity and live birth weight.

Wider implications of the findings: By accounting for the concealing effect of multiple confounders, we associated, for the first time, EMT to a greater than expected influence on IVF outcomes, specifically LBR. These results reestablish the role for EMT assessment in modern-day IVF, where cycle monitoring and elective embryo cryopreservation may adequately circumvent this issue.

Trial registration number: Not applicable.

O-112 Is endometrial thickness associated with intrauterine growth restriction or placental related pregnancy complications in fresh IVF cycles?

G. Oron[1], S. Rona[1], O. Sapir[1], L. Hirsch[1], Y. Shufaro[1], B. Fisch[1], A. Ben-Haroush[1]
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Study question: Our objective was to evaluate the association between endometrial thickness, on day of hCG trigger in fresh IVF cycles and intrauterine growth restriction (IUGR) and placental related obstetric complications.

Summary answer: Univariate analysis shows that a thin endometrial thickness (<8 mm) is associated with increased risk of intrauterine growth restriction and grouped placental-related pregnancy complications.

What is known already: IVF pregnancies in general appear to be associated with worse obstetric and perinatal outcomes such as preterm delivery and low birth weight compared to spontaneous conceptions. A previous study has shown that a thick endometrial lining (>12 mm) is associated with an increased risk for placenta previa. We conducted a study evaluating the association between endometrial thickness and the resulting neonatal birth weight and placental related pregnancy complications.

Study design, size, duration: A retrospective analysis of 1,002 consecutive singleton live births from a cohort of 6,350 fresh ET cycles performed between July 2007 and December 2014. Data for confounding variables were available for 849 singleton deliveries. Intrauterine growth restriction was defined as <10% according to a national population-based live born infant birth weight curves.

Participants/materials, setting, methods: We analyzed patient variables (maternal age, smoking, BMI), treatment cycle parameters (stimulation protocol, total gonadotropin dose, ICSI/IVF) and pregnancy outcome (mode of delivery, gestational age, birth weight and pregnancy complications). Composite placental related pregnancy complications included preeclampsia and pregnancy induced hypertension, placenta previa, placental abruption and intrauterine growth restriction)

Main results and the role of chance: Univariate analysis shows a significant association between a thin endometrium (<8 mm) and intrauterine growth restriction (35/199, 17.6% and 67/650, 10.3%; $p = 0.006$). Thin endometrium was significantly associated with composite adverse outcome (19.9 and 12.8%, $p = 0.014$). In a multivariate logistic regression analysis, adjusting for confounding factors including age, smoking, BMI, parity, chronic hypertension, pre-gestational diabetes and gestational diabetes, endometrial thickness was not a significant predictor of intrauterine growth restriction or placental-related pregnancy complications.

Limitations, reasons for caution: The limitation of the study is the retrospective nature of the study. Medical charts were reviewed for patients who delivered at our hospital. Pregnancy outcome was recorded as part of routine prospective patient follow-up by telephone surveillance and documented in the patient chart for patients that delivered out of hospital.

Wider implications of the findings: Univariate analysis shows a strong association between endometrial thickness and placental-related complications and IUGR. When adjusting for maternal risk factors such as advanced age, smoking, BMI, chronic hypertension and pre-gestational diabetes this effect is non-significant. Further larger studies are needed to dispute or confirm these findings.

Trial registration number: None.

O-113 ER map: a new comprehensive and reliable endometrial receptivity test

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[4]*IVF Spain, Reproductive Medicine, Alicante, Spain*
[5]*Reprogenetics, Research, Livingston, NJ, USA*

Study question: Is it possible to determine the receptivity status of an endometrium by combined qRT-PCR expression analysis of genes involved in endometrial proliferation and immune response?

Summary answer: The new ER Map test can predict endometrial receptivity status by qRT-PCR using a panel of 16 genes involved in endometrial proliferation and immune response.

What is known already: The endometrium reaches a receptive status for embryonic implantation around day 19–21 of the menstrual cycle. During this period, known as the window of implantation (WOI), the endometrium shows a specific gene expression profile suitable for endometrial function evaluation. The number of molecular diagnostic tools available to characterize this process is very limited and lack key elements for the accurate determination of the WOI, such as immune response genes, crucial for embryo implantation. In this study, qRT-PCR analyses of genes involved not only in endometrial receptivity development but also in the immune response metabolism is performed for the first time.

Study design, size, duration: A comprehensive solution to analyse the endometrial transcriptomic signature at the WOI is explored. ER Map validation was achieved on 130 endometrial samples including fertile women and patients undergoing fertility treatment between July 2014 and December 2015. Expression analyses of 192 genes involved in endometrial receptivity and immune response were performed. All patient samples were additionally tested with an independent endometrial receptivity assay (ERA) to verify their endometrial status.

Participants/materials, setting, methods: A total of 96 fertile women (18–34 y.o) and 34 ART patients participated in the study. Endometrial biopsy samples were obtained at LH + 2 and LH + 7 in fertile subjects and at P + 5 (progesterone) in patients. Total RNA was purified using RNeasy Mini Kit (Qiagen) and quality-checked using Agilent Bioanalyzer. Oligonucleotides for the amplification of the 192 selected genes were designed by using GeneFisher 2.0 platform. Gene expression was quantified by qRT-PCR using Biomark-HD platform (Fluidigm).

Main results and the role of chance: The new ER Map test can predict endometrial receptivity status by qRT-PCR using a new panel of 16 genes involved in endometrial proliferation and immune response. Mean gene expression of 96 out of the 192 selected genes was found to be statistically different when comparing LH + 2 and LH + 7 samples (*T*-test, $p < 0.05$). Those genes were mainly related to the cell division, tissue proliferation and immune system metabolism. Principal Component Analysis showed that more than 90% of the gene expression variance of the set of samples studied was explained by 16 key genes. These genes allowed accurate classification of samples into 4 endometrial receptivity status: non-receptive, pre-receptive, receptive and post-receptive in both groups, fertile women and infertile patients. Using a discriminant model based on the 16 selected genes, 100% cases were correctly classified. In the group of fertile donors, 100% of LH + 2 samples were categorised as non-receptive, and all LH + 7 samples were classified as receptive. Within the patient group, ER Map classification matched the endometrial biopsy status prediction of the independent diagnostic tool ERA in all 100% samples.

Limitations, reasons for caution: A higher number of samples are desirable to fine receptivity status prediction and minimise no results. A clinical trial to evaluate IVF treatment success following ER Map application is required to assess the advantages of this new test for accurate prediction of the WOI and improvement of implantation.

Wider implications of the findings: A new comprehensive system for human endometrial evaluation based on receptivity and immunology-linked genes has been developed. This molecular tool has been optimised in the diagnostic

platform, BioMark-HD (Fluidigm) that allows high-throughput gene expression analyses at a lower cost than other methods.
Trial registration number: Not applicable.

O-114 How to limit the risk of early pregnancy loss in frozen embryo transfer protocols?

I. Hatoum[1], L. Bellon[1], M. Ouazana[1], N. Swierkowski[1], S. Bouba[1], K. Fathallah[1], B. Paillusson[1], M. Bailly[1], F. Boitrelle[2], L. Alter[2], M. Bergère[2], J. Selva[2], R. Wainer[1]
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[2]*Biology and Cytogenetics Laboratory of the ART Unit, Obstetrics and Gynecology, Poissy, France*

Study question: Does the cycle regimen used for endometrial preparation affect the endometrial receptivity and thus the reproductive outcomes in frozen embryo transfer (FET)?
Summary answer: The early pregnancy loss rate increases when using artificial cycles (estrogen E2 and progesterone P) for endometrial preparation compared to stimulated cycles (follicle-stimulating hormone FSH) in FET.
What is known already: When comparing live birth and clinical pregnancy rates, a Cochrane review in 2010 (natural, stimulated and artificial cycles) (1), a meta-analysis published in Human Reproduction Update 2013 (2) and a retrospective study by Tomas et al.(3) (natural cycle with or without ovulation induction and artificial cycles) could not demonstrate the superiority of one regimen over another. But according to Tomas et al., there was a significantly higher early pregnancy loss ($P < 0001$) in the artificial cycles group. Only the type of protocol used correlated to the pregnancy loss (3). Moreover the optimal endometrial thickness for FET is yet to be defined because of controversies (1, 4).
Study design, size, duration: Our retrospective comparative study included 1,926 FET cycles performed during a period of 3.5 years (January 2012–June 2015) in the Fertility unit of the intercommunal hospital center of Poissy-Saint-Germain-en-Laye in France. The outcomes of assisted reproductive technology in terms of clinical pregnancy and early pregnancy loss between January 2012 and June 2015 at a single institution were retrospectively evaluated. The embryos were derived from either *In Vitro* Fecondation or intracytoplasmic sperm injection treatment cycles.
Participants/materials, setting, methods: We retrospectively evaluated clinical pregnancy and pregnancy loss rates in 1,926 cycles (substituted cycles: estrogen 4–6 mg daily orally or vaginally followed by vaginal progesterone (P) supplementation 400–600 mg daily before embryo transfer and until the 12th gestational week if pregnancy occurred) and stimulated cycle with FSH from day 4, followed 2 days after ovulation triggering by a measure of progesteronemia). Vaginal ultrasound was used for endometrial and ovarian monitoring. The pregnancy test was performed 14 days after ET.
Main results and the role of chance: Before embryo transfer ET, a substituted cycle was used for endometrium preparation in 865 cases (45%) whereas a stimulated cycle was used in 1,061 ET (55%). Baseline characteristics (age, body mass index, duration of infertility, etiology of infertility and number of embryo transferred) were similar in the two groups. According to Guidelines for clinical practice by the French College of Gynaecologists and Obstetricians in 2014 a miscarriage is defined by the loss of pregnancy <14 weeks after a clinical pregnancy was identified. A biochemical pregnancy is defined by a rate of BHCG < 100. We define early pregnancy loss (EPL) as biochemical pregnancies (preclinical losses) and miscarriages. EPL rate was 34.2% in stimulated cycles vs. 56.9% in substituted cycles.

Independently of patient's age, BMI, duration of infertility and number of embryo transferred, substituted cycles had an increased risk of miscarriage nearly 2 times that of stimulated cycles while live birth rate is significantly higher (2 times) in stimulated cycles than in substituted cycles (59.7 vs. 29.1% respectively).
Limitations, reasons for caution: The compliance of patients to treatment in artificial cycles is important for analysis of these findings in term of efficacy, even if patients are given written prescription to continue hormonal supplementation to 12 weeks. Due to retrospective nature of our study selection or information bias could be found.
Wider implications of the findings: Stimulated cycles provide a "physiologic" hormonal support *via* the corpus luteum that could lead to more live births and less early pregnancy loss in FET candidates but should be carefully used in ovarian polycystic syndrome. A prospective randomized study is needed to a better analysis of the efficacy of protocols.
Trial registration number: 0

O-115 Enhanced SUMOylation of HOXA10 impairs endometrial receptivity and embryo implantation

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Study question: Does SUMOylation of homeobox a10 (HOXA10) regulate the endometrium receptivity and embryo implantation?
Summary answer: HOXA10 SUMOylation at K164 impairs endometrium receptivity and embryo implantation through decreasing protein nuclear localization and repressing transcriptional activity.
What is known already: HOXA10 plays a critical role in the endometrium receptivity and embryo implantation by regulating the expression of downstream target genes, including β3-integrin (ITGβ3) and matrix metalloproteinase-26. Recently, the acetylation of HOXA10 has been demonstrated to impair embryo implantation by inhibiting ITGβ3 protein expression.
Study design, size, duration: The mid-secretory phase endometrium tissues from fertile controls ($n = 22$) and patients with recurrent implantation failure (RIF) ($n = 22$) were collected for investigation of SUMOylation level. HEK293T and Ishikawa cells were used to investigate the molecular mechanisms *in vitro* analysis. This is an experiment study lasted 1 year.
Participants/materials, setting, methods: HEK293T and Ishikawa cells were used as study models. A luciferase reporter assay, Western blotting, quantitative PCR and confocal immunofluorenscence analysis were used to determine the effect of small ubiquitin-like modifier 1 (SUMO-1) on HOXA10 protein nuclear localization and transcriptional activity. The effect of HOXA10 SUMOylation on embryo implantation was evaluated using a BeWo spheroid attachment assay with adenovirus-mediated overexpression of HOXA10 and SUMO-1.
Main results and the role of chance: We found increased SUMO-1 conjugation to substrate proteins with 50–90 KD molecular weight in the endometrium of patients with RIF compared to fertile controls ($P < 0.05$). HOXA10 was covalently modified by SUMO-1 on a phylogenetically conserved lysine 164 and increased amount of SUMO-1 expression led to progressive increment of SUMOylated HOXA10. A combination of estrogen and progesterone stimulation in Ishikawa cells contributed to decreased SUMOylated HOXA10 level. HOXA10 protein (Wild Type) was expressed preferentially in the nucleus as expected, while the SUMOylation deficient K164R mutant protein was prevalently localized in the cytoplasm. SUMO-1 modification attenuated the HOXA10-mediated ITGβ3 transcription by 35% ($P < 0.01$). Interestingly, HOXA10 mutant (K164R) had high basal transcriptional activity. Furthermore, the adenovirus-mediated overexpression of HOXA10 and SUMO-1 in Ishikawa cells markedly diminished BeWo spheroid adhesion by 33% compared to the HOXA10 alone ($P < 0.05$).
Limitations, reasons for caution: This is an *in vitro* study utilizing Ishikawa and 293T cells. The effect of SUMOylation on HOXA10 protein stability and its relationship with ubiquitylation were not evaluated. Furthermore, the results should be confirmed in a mouse model of overexpressed HOXA10 mutant (K164R) in endometrium *in vivo*.
Wider implications of the findings: Our identification of SUMOylation as a novel post-translational modification of HOXA10 suggests SUMOylation plays an important role in endometrium receptivity and embryo implantation, and this may subsequently provide novel insights to our comprehensive understanding about the pathological physiological mechanisms of embryo implantation.
Trial registration number: None.

O-116 Impact of S100A10 protein silencing on the decidualization process and secretory transformation of endometrial stromal and epithelial cells, of fertile women

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Study question: Determine whether a human endometrial receptivity biomarker (S100A10) plays a role in the decidualization and secretory transformation of endometrial cells in normal responder patients.
Summary answer: S100A10 protein silencing inhibited prolactin secretion in stromal endometrial cells, but significantly enhanced it in epithelial endometrial cells.
What is known already: The role(s) of human biomarkers of endometrial receptivity during the implantation window are rarely determined. Stromal cells decidualization and epithelial cells secretory transformation are critical for the establishment of uterine receptivity, and consequently, for successful implantation. Decidualization characterizes the differentiation of endometrial stromal fibroblasts into specialized secretory decidual cells that expressed specific markers such as prolactin. Decidualization is endowed with the secretory transformation of the uterine glands. As for decidualization, uterine gland maturation is mediated through cAMP allowing expression of implantation-related factors including the prolactin. The behaviour of epithelial cells during the decidualization of stromal cells has rarely been studied.
Study design, size, duration: Primary epithelial and stromal cells were purified from endometrial biopsies obtained during the implantation window of two fertile patients. Then, we targeted the extinction of the S100A10 using shRNA in each endometrial cell type. The obtained phenotype was analyzed in regards of the decidualization and the secretory transformation of stromal and epithelial cells respectively.
Participants/materials, setting, methods: Primary endometrial cell cultures of epithelial and stromal cells were performed. Then, to determine whether S100A10 had a role in the decidualization of endometrial stromal cells and the secretory transformation of epithelial cells, each cell type transduced with S100A10 shRNA or control shRNA were incubated or not with 8-Br-cAMP for 9 days. Spent culture medium was collected for prolactin quantification with the automated immunoassay system BRAHMS KRYPTOR.
Main results and the role of chance: The incubation with 8-Br-cAMP of the stromal and epithelial endometrial cells transduced with control shRNA, induced a typical decidual phenotype and prolactin secretion. In the stromal cells, prolactin secretion was increased by 16.3-fold in the culture medium of 8-Br-cAMP treated cells transduced with control shRNA compared with untreated cells (215 ± 33 vs. 13 ± 1 µUI/ml, $P < 0.0001$) and only by 8.3-fold in S100A10 silenced cells (110 ± 10 µUI/ml, $P = 0.009$) compared to control shRNA. These data indicate that for the stromal endometrial cells there is an inhibitory effect of prolactin secretion in S100A10 silenced cells. In endometrial epithelial cells, 8-Br-cAMP treatment induced prolactin secretion in both cells transduced with control shRNA (62 ± 11 vs.13 ± 1 µUI/ml, $P < 0.0001$) compared to untreated cells and with the S100A10 shRNA (98 ± 10 µUI/ml, $P = 0.025$) compared to control shRNA, indicating that in epithelial endometrial cells, S100A10 silencing promotes prolactin secretion. Therefore, S100A10 silencing inhibited prolactin secretion in stromal endometrial cells, but significantly enhanced it in epithelial endometrial cells.
Limitations, reasons for caution: Culture of luminal and glandular epithelial cells, as well as co-culture system between epithelial and stromal cells, should be considered to better understand cross-talk between these cellular compartments.
Wider implications of the findings: This study should open new perspectives in the understanding of molecular mechanisms regulating human endometrial receptivity and reveals the key role of S100A10 as a player in endometrial receptivity acquisition.
Trial registration number: Not applicable.

SELECTED ORAL COMMUNICATIONS

SESSION 33: DEEP SEQUENCING THE EMBRYO.

Tuesday 05 July 2016 **Hall 3 AB** **10:00–11:30**

O-117 Quantification of low frequency variants of the mitochondrial DNA (mtDNA) in single cells by massive parallel sequencing (MPS)

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Study question: Is it possible to detect and quantify low frequency heteroplasmic variants, large deletions and single nucleotide variants (SNV) in the mtDNA of single cells?
Summary answer: Our method detects heteroplasmic SNVs and large deletions in single cells; data on single oocytes provides interesting insight on SNV segregation during oogenesis
What is known already: The advent of MPS has opened new possibilities for the analysis of low frequency heteroplasmies in the mtDNA, but no methods are currently available to accurately detect and quantify low frequency SNVs and large deletions. Mutations in the mtDNA have been previously reported in ART-derived oocytes and embryos, as well as methods for the analysis of individual mutations in preimplantation genetic diagnosis. Conversely, up to now, it has not been possible to identify all mutations in one single cell simultaneously, nor to establish their mutation load
Study design, size, duration: We developed a method to analyze large deletions and SNVs in DNA samples and scaled it down to the level of single cells. We sequenced six single fibroblasts from a control individual, two muscle fibers from a patient with mtDNA deletions, and 11 oocytes from three different patients treated at our IVF center (5 GV, 3 MI, 3 MII)
Participants/materials, setting, methods: Single cells were lysed in alkaline lysis buffer. MtDNA was enriched by long-range PCR. Sequencing was done at 6,000×. SNVs called with CLCBio Genomic Workbench and large deletions were identified using BLAST. Mutation load was established using the sequencing depth. The lower detection limits are linked to the number of PCR cycles needed, down to loads for deletions and SNVs of 0.5 and 1.5% at 35 cycles, and 0.5 and 2% at 45 cycles, respectively
Main results and the role of chance: The single fibroblasts revealed somatic mosaicism, with two variants appearing in 1/6 of the cells at high loads, and not present in the other cells. The muscle fibers showed a low number of large deletions at a high frequency and most of them were also identified in the blood sample of the same patient. In the oocytes, one large deletion at 0.51% was found in the cohort analyzed. We detected SNVs in 9/11 oocytes, at frequencies ranging from 1.5 to 20%. The same SNVs were found recurrently in the oocytes from the same donor but often at different frequencies. In one patient we observed a variant in 3/4 oocytes at loads of 5, 15 and 20%. In the same patient, another variant was detected in 2/4 oocytes at frequencies of 5 and 15%. In 2/4 oocytes from a different patient, a third SNV was detected at frequencies of 2.5 and 3%. Half of the detected variants mapped to the hypervariable region (12/24) and only one variant at load of 4% was predicted to be pathogenic. There was no correlation between the oocyte maturation stage and the presence of variants
Limitations, reasons for caution: The results we obtained with the oocytes must be matched with the variants in somatic tissues of the same patient to have a full validation of the variants called and to follow the variants' segregation
Wider implications of the findings: Our method for low frequency detection can be used to study mtDNA variants in single cells with high accuracy. This tool can be applied also to the diagnostic of mitochondrial diseases involving large deletions or SNVs. Finally, our data provides interesting preliminary information about heteroplasmy shifts during gametogenesis.
Trial registration number: N.A.

O-118 Design, validation and clinical application of a novel next-generation sequencing protocol for detection of mitochondrial disease and simultaneous aneuploidy screening in preimplantation embryos

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Study question: Can a next-generation sequencing (NGS) protocol be clinically applied to perform preimplantation genetic diagnosis (PGD) of mitochondrial DNA (mtDNA) disease and aneuploidy in preimplantation embryos?
Summary answer: The NGS technique enables accurate PGD of mtDNA disease in cleavage- and blastocyst-stage biopsies. Additionally, comprehensive chromosome screening (CCS) can be incorporated in the protocol.
What is known already: Maternally inherited mutations can be present in all (homoplasmy) or a fraction (heteroplasmy) of mtDNA copies. Although most female carriers are phenotypically unaffected, due to low mutation load, high levels can be present in oocytes and passed to the offspring resulting in mtDNA disease. To prevent transmission, PGD can be performed to select embryos free of the mutation or unlikely to be affected by disease. Current PGD techniques of mtDNA disease do not allow incorporation of CCS. Since aneuploidy is extremely common in preimplantation embryos, we aimed to develop a test that enables accurate mtDNA mutation detection and aneuploidy screening.
Study design, size, duration: To combine mtDNA mutation and aneuploidy screening, the technique was developed and optimised on whole genome amplified (WGA) DNA. This was then subjected to targeted sequencing of the mtDNA for PGD and to microarray comparative genomic hybridisation (aCGH) or NGS for the purpose of CCS. Prior to clinical application, the protocol was extensively validated on genomic DNA and isolated buccal cells and/or lymphocytes, derived from four mtDNA mutation carriers presenting varying mutation levels (50–100%).
Participants/materials, setting, methods: The method was applied to developmentally competent and arrested embryos and unfertilised oocytes from three patients, each having a child affected by Leigh Syndrome (Patient 1 and 2: m.8993T > G; Patient 3: m.10191T > C). The mutation was not detectable in somatic cells of Patients 1 and 3, however children of both presented with heteroplasmy (95 and 78% mutant mtDNA, respectively). Somatic heteroplasmy of 56% was detected in Patient 2 who had a child homoplasmic for the mutation.
Main results and the role of chance: The NGS method provided qualitative (DNA sequence) information and also quantitative data (proportion of mtDNA molecules with the mutation). Initial validation using genomic DNA and cells of mutation carriers yielded 100% diagnostic accuracy. Five PGD cycles were performed with 50 specimens analysed: 19 blastocyst- and cleavage-stage embryos, three unfertilised oocytes, eight arrested embryos. Additionally, three embryos were divided into 20 single sister blastomeres. Patient 1 generated 14 blastocysts. None carried detectable mutation levels, but four were aneuploid. No mutations were detected in one unfertilised oocyte, one arrested embryo and 16 sister blastomeres from two embryos. The patient underwent a single embryo transfer and is pregnant (delivery in May). Patient 2 generated one blastocyst after two cycles, which was unaffected by the mutation, but aneuploid. One unfertilised oocyte and 2 arrested embryos carried 91, 89 and 0% of mutated mtDNA copies, respectively. Patient 3 produced four embryos from two cycles, which were assessed at the cleavage-stage. None carried the mutation, but two were aneuploid. No mutation was detected in one unfertilised oocyte, four arrested embryos and four sister blastomeres of one embryo. Transfer of a single embryo yielded no pregnancy and the patient is awaiting transfer of the second euploid embryo.
Limitations, reasons for caution: The accuracy of PGD for mtDNA mutation analysis may be reduced due to possible biological variations in mutation levels between the biopsy specimen and the remaining embryo. Hence verification by prenatal testing should be performed to confirm that the fetus is not affected by mtDNA disease.
Wider implications of the findings: This is the first study utilising NGS for the detection and quantification of mtDNA mutations in preimplantation embryos. The protocol was to be demonstrated highly accurate and can be combined with chromosome screening. The study provided evidence of low recurrence risk of mtDNA disease in patients with undetectable somatic mutations.
Trial registration number: NA.

O-119 Mitochondrial quantification during human embryogenesis *via* next-generation sequencing: relation to nuclear ploidy status

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Study question: What is the association between embryo mitochondrial load and nuclear ploidy status during early human embryo development?
Summary answer: Mitochondrial quantity appears to vary throughout the sequence of human embryo development and does correlate with nuclear ploidy status.
What is known already: Mitochondria are recognized as the critical source of cellular ATP although they are also involved in redox & calcium homeostasis, provide intermediary metabolites, and store proapoptotic factors. They are maternally transmitted, deriving from a restricted founder population amplified during oogenesis. While mitochondrial segregation to blastomeres during early embryogenesis is strictly regulated, the distribution of mitochondria during this critical phase remains poorly understood.
Study design, size, duration: This retrospective, multi-cohort pilot study was undertaken to quantify mitochondrial load during early embryogenesis and to assess the relation of mtDNA to nuclear ploidy status. Data from embryos (n = 325) were reviewed for patients undergoing IVF at a single institution during the 9 month interval ending December 2015.
Participants/materials, setting, methods: Embryo records were evaluated from patients (n = 69) who completed IVF treatment with preimplantation screening at an urban referral fertility unit in southern California. High throughput sequencing of mitochondrial and nuclear DNA was achieved simultaneously after whole genome amplification using an ion semiconductor platform (ThermoFisher Scientific, Inc). Nuclear and mitochondrial DNA ratios were then tabulated according to time of single biopsy: day 3 (n = 90), day 4 (n = 145), or day 5 (n = 90).
Main results and the role of chance: Successively declining mitochondria quantities were observed from the blastomere stage (d3) compared to morula (day-4) and blastocyst stage (day-5), validating a prior observation that mitochondria are not newly made during the first 5 days of human embryo development. Among euploid embryos, we noted a relatively high mitochondria load at d3 but successively fewer mitochondria at d4 and d5 vs. aneuploid embryos.
Limitations, reasons for caution: Absolute quantity of mitochondria may not always correlate to mtDNA copy number estimates, so additional study is planned to measure discrete mitochondrial parameters at our center in the future. These data also cautiously align with previous work in that fewer mitochondria were present in embryos obtained from older IVF patients.
Wider implications of the findings: We hypothesize that the observed decline in mitochondrial load at d4 and d5 among euploid embryos results from an enhanced metabolic milieu, where less ATP is needed to execute vital cellular processes. Additionally, impaired cell fission may allow aggregation of mtDNA that would otherwise be dispersed in "healthy" embryo cells.
Trial registration number: N/A.

O-120 The impact of simultaneous mitochondrial DNA (mtDNA) content assessment in comprehensive chromosomal screening (CCS): a prospective pilot study

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Study question: Does simultaneous mtDNA copy number assessment bring additional benefit on cycle outcome in frozen embryo transfer cycles where euploid blastocysts are replaced after CCS?
Summary answer: Our study shows a high correlation between mitochondrial DNA copy number assessment and implantation potential of euploid human embryos in CCS cases.

What is known already: Recent studies indicate that, besides its developmental stage and morphology, successful implantation after the transfer of a chromosomally normal human embryo is highly correlated with its low mtDNA content. On the other hand, although the quantity of mtDNA is found to be higher in older women and is associated with aneuploidy, the data regarding its clinical relevance in euploid embryos is scarce.
Study design, size, duration: This prospective pilot study was performed in Bahçeci Fulya IVF Centre between May and July 2015. It includes 120 blastocyst-stage human embryos from 38 patients undergoing consecutive CCS cycles for advanced maternal age, recurrent implantation failure, recurrent first trimester abortions or a combination of these indications.
Participants/materials, setting, methods: Whole genome amplification products of 52 embryos that were found to be euploid (52/120; 43.3%) after CCS were further processed for mtDNA content assessment (Mitoscore analysis) by qPCR. Embryo transfers were performed prospectively and blinded for Mitoscore results (by using CCS results only). Clinical outcome data and individual Mitoscore results of each embryo transferred have been paired and analyzed retrospectively with the known implantation data (KID) at the end of the study.
Main results and the role of chance: Of 52 embryos analyzed by Mitoscore, 24 (46.2%) were found to have high (Group A), 26 (50.0%) were found to have average (Group B) and 2 (3.8%) were found to have low (Group C) implantation potential respectively according to a previous validation study. In 30 embryo transfer cycles, uterine replacement of 31 mitoscore-tested blastocysts resulted in 53.3% clinical pregnancy rate. When the same data were analyzed according to Mitoscore groups, clinical pregnancy and implantation rates were found to be 69.2% and 71.4% for Group A and 46.6% and 53.3% for Group B embryos respectively. There were two embryos in Group C and both embryos failed to create a viable pregnancy after uterine replacement. Also, no correlation was found among blastocyst morphology, day of trophectoderm biopsy and Mitocsore results.
Limitations, reasons for caution: Although a higher pregnancy and implantation rates were observed with embryos with low mtDNA content, this study should be expanded to include larger sample sizes with an adequate statistical power. Such work is currently under way in the Authors' institutions.
Wider implications of the findings: The results of this pilot study, when confirmed in a wider study population will modify our current CCS concept and create a platform in which mtDNA-based analyses can become an integral part of the CCS procedures in order to maximize the clinical outcome.
Trial registration number: None.

O-121 RNA-Seq reveals distinct transcriptomes in normal and trisomic human pre-implantation embryos

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Study question: What do RNA seq-profiles tell us about gene activity in pre-implantation embryos known to be normal vs. trisomic for chromosomes 21, 18, 15 and 22.
Summary answer: RNA-Seq profiles are karyotype-dependent and appear to correlate with viability. X and Y transcription is surprisingly active at the pre-implantation stage.
What is known already: Embryonic karyotypic abnormalities are the most common cause of implantation failure and miscarriage. Some such embryos can progress to viability as evidenced by the birth of children with Trisomy 21, 18 and 15. An abnormal karyotype dictates developmental aberrations, and for these differences to affect phenotype, variations in gene expression must occur in the developing embryo. We set out to determine if such changes could be identified in embryos as early as the pre-implantation stage.
Study design, size, duration: We used a cohort of 19 pre-implantation embryos (day 5 and 6 blastocysts); three being normal, five trisomy 15, two trisomy 22, three trisomy 21 and three trisomy 18, and three of unknown karyotype.
Participants/materials, setting, methods: After written consent, analysis was performed on high quality embryos that previously underwent trophectoderm biopsy with array comparative genomic hybridization (aCGH) or next generation sequencing (NGS) for 24-chromosome aneuploidy screening and vitrified. Blastocysts were lysed immediately after thawing, and cDNA was synthesized and amplified, followed by RNA-Seq library preparation for deep Illumina-based sequencing. Sex and chromosomal aneuploidy were used as parameters for comparative analysis using a bioinformatics pipeline using a sensitivity of 0.05.
Main results and the role of chance: Principal Component analysis (PCA) revealed that normal embryos clustered in proximity to trisomies 21 and 18, while 15 and 22 clustered separately. This suggested that early gene expression may correlate with viability. PCA did not distinguish between male and female embryos. Differential gene expression was calculated using DESEQ2, an R package that estimates the variance-mean dependence and tests for differential expression using a model using the negative binomial distribution. A comparison of sex-specific gene expression showed that the top differentially expressed genes were on the sex chromosomes, including various Y-linked transcription factors, helicases, and ribosomal proteins, as well as a testis-specific regulatory transcript, and >200 X-chromosome genes. Trisomy 21 embryos were the closest to normal embryos, with only 3 genes on chromosome 21 expressed more highly in the trisomic embryos. In contrast, trisomy 22 embryos (non viable), had 684 differentially expressed genes, 32 of which on chromosome 22. Trisomy 18 embryos had only one highly expressed significant gene, and it is located on chromosome 18. Trisomy 15 embryos had 829 differentially expressed genes, of which 83 were in chromosome 15. Our results suggest that the less viable trisomies have bigger gene expression differences, even at this very early stage.
Limitations, reasons for caution: While the karyotypes were analyzed by stringent methods, embryos may have had elements of mosaicism or chromosomal structural abnormalities. The genes identified by RNA-Seq need to be validated using orthogonal methods.
Wider implications **of the findings:** We have expanded the knowledge of the transcriptome of the human pre-implantation embryo as they relate to aneuploidy and sex. This information can now be used to further our understanding of early embryonic development and stem cell biology, and to identify biomarkers for non-invasive preimplantation genomic screening.
Trial registration number: NA.

O-122 Selecting single blastocysts for transfer with next-generation sequencing significantly improves ongoing pregnancy rates for IVF patients: results from a randomized pilot study

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Study question: Can next-generation sequencing (NGS) be used efficiently for selecting single blastocysts for transfer in patients undergoing IVF treatments?
Summary answer: Our data demonstrate that selection of single blastocysts for transfer with NGS screening results in significantly higher clinical and ongoing pregnancy rates for IVF patients.
What is known already: Early randomized clinical trials with FISH screening of a limited numbers of chromosomes resulted in disappointing pregnancy outcomes. Recent studies with aCGH screening of 24 chromosomes have resulted in a significant increase in ongoing pregnancy and implantation rates for IVF patients. More recent advances in next-generation sequencing have provided new methods for screening embryos from IVF cycles. However, there is still limited information about clinical application of NGS in selecting single blastocysts for transfer in a randomized study.
Study design, size, duration: A total of 204 IVF patients at mean age 31.2 ± 3.6 years were enrolled in this prospective randomized pilot study in our multiple IVF clinics from March 2014 to March 2015. The cohort patients met the inclusion criteria for enrollment in this study and singed consents for selecting single embryos for transfer in order to avoid multiple pregnancies.
Participants/materials, setting, methods: The enrolled patients were randomized into NGS (Group A, n = 103) and non-NGS control (Group B, n = 101). For both groups, all embryos were cultured to blastocyst stage. Blastocysts were vitrified after biopsy on day 5 (up to 10:00 p.m.). A single euploid blastocyst was selected for transfer to individual patients based on NGS results in Group A and morphological assessment alone in Group B.

Main results and the role of chance: This is the first randomized clinical study on the efficiency of NGS for selecting single embryo for transfer in IVF patients to avoid multiple pregnancies. Data analysis revealed that the demographic parameters of the enrolled patients in Group A and Group B were similar ($p > 0.05$). The fertilization and blastocyst rates were also comparable between the two groups ($p > 0.05$). Clinical pregnancy rate was significantly higher in Group A compared to group B (70.8 vs. 47.5%, respectively, $p < 0.05$). The observed ongoing pregnancy rate in Group A was also higher than that of Group B (69.9 vs. 45.5%, respectively, $p < 0.05$). There were no multiple pregnancies in both groups.
Limitations, reasons for caution: Although NGS brings distinct clinical benefits for many IVF patients, the approach is not for all patients, especially those with diminished ovarian reserve and with balanced translocations. Further randomized studies with a larger sample are needed to define the role of NGS for all age groups of IVF patients.
Wider implications of the findings: With the resulting high ongoing pregnancy rates following transfer of the screened embryos, NGS has demonstrated an efficient, robust high-throughput technology for selecting single blastocysts for transfer. With the enhanced capability of detecting segmental imbalances, NGS may detect aneuploidy and imbalance translocations at the same time.
Trial registration number: No applicable.

SELECTED ORAL COMMUNICATIONS

SESSION 34: REPRODUCTIVE EPIDEMIOLOGY, SOCIO-CULTURAL ASPECTS AND HEALTH ECONOMY 1

Tuesday 05 July 2016 **Hall 3 DE** **10:00–11:30**

O-123 Fewer live-births with repeated single embryo transfer as compared to initial double embryo transfer; time to question the efficacy of embryo cryopreservation?

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Study question: What is the cumulative live-birth rate, multiple birth rate and number of infants born comparing two single embryo transfers (SET) to one double transfer (DET)?
Summary answer: Cumulative live-birth rates were equivalent after initial eSET or DET in women <37 years but fewer infants were born to all women with repeated eSET.
What is known already: Observational data from national registries and four randomised controlled trials ($N = 42–661$) suggest equivalent live-birth rates of one or more live-births, when repeated SET is compared to DET, but with a markedly lower multiple pregnancy rate. That the RCTs reported fewer total infants born from a repeated eSET strategy as compared to the DET suggests that elective embryo cryopreservation may adversely impact on embryo viability.
Study design, size, duration: Prospective study of 135,950 UK women who received 199,204 IVF ovarian stimulation cycles and 249,602 embryo transfers between 2003 and 2010. Main exposure *in vitro* fertilization, with a cycle defined as an episode of ovarian stimulation and all subsequent separate fresh and frozen embryo transfers.
Participants/materials, setting, methods: We used data on all ART events occurring in the United Kingdom between 1 January 2003 and 31 December 2010, followed up until 30 June 2012, with linkage of cycles to individual women and birth outcomes. We excluded all women with fewer than two embryos available for transfer. Live-birth was defined as one or more infants were born alive after 24 weeks' gestation and surviving longer than 1 month from a singleton or multiple pregnancies.
Main results and the role of chance: The odds of a live-birth were similar between initial SET and initial DET in patients aged less than 37 years or using donor oocytes (adjusted odds ratio 0.98, 95% CI 0.96, 1.01), with a lower live-birth rate with SET in 37- to 39-year olds (adjusted OR 0.79, 95%CI 0.72, 0.86) and in >39-year-olds (adjusted OR 0.60, 95%CI 0.51, 0.69). Comparing the two strategies for the use of the first two embryos available demonstrated that initial transfer of a single embryo and then a single frozen embryo had an equivalent unadjusted live-birth rate (30.8% (6,548 live-births, 6,643 infants from 21,261 cycles) as observed after initial fresh transfer of two embryos (30.9% (55,012 live-births, 70,018 infants from 177,943 cycles)), with a substantially lower risk of multiple pregnancy and number of infants born. Thus the number of infant rates differed between the two approaches (31 vs. 39%) and if we anticipated both groups should have similar infant rates from their first two embryos there are 1,649 fewer infants in the eSET group.
Limitations, reasons for caution: Given the time frame analysed cleavage stage embryos transfers dominated and only 12.7% of SET replacements that did not result in a live birth were followed by a frozen SET replacement.
Wider implications of the findings: Although the overall live-birth rates were equivalent after repeated eSET or DET in young women, fewer infants were born in all women with use of eSET. This suggests that the efficacy of embryo freezing may have been overestimated.
Trial registration number: –

O-124 Use of fertility treatments among female survivors of early onset cancer – a Finnish register-based study

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Study question: Do female early onset cancer survivors giving birth have more fertility treatments compared to female siblings without a history of cancer?
Summary answer: Female cancer survivors giving birth have a statistically increased risk for fertility treatments compared to female siblings.
What is known already: The relative probability of parenthood in former early onset cancer patients has been reported to be reduced by 50% compared to their siblings. Previous studies have shown that female cancer survivors have an increased risk of infertility, especially after radiotherapy and chemotherapy with alkylating agents. One previous study, based on self-reported outcomes, showed female survivors to have more need of fertility treatments than their siblings.
Study design, size, duration: In this retrospective register-based study, nationwide cancer and birth registries were merged to identify 1,974 post diagnosis births of female cancer survivors and 6,107 births of female siblings between January 2004 and December 2013. The birth registry provided information on possible fertility treatments initiating these pregnancies.
Participants/materials, setting, methods: Cancer survivors and siblings with either a pregnancy continuing for at least 22 weeks or a fetus weighting over 500 g were identified from the birth registry. Unconditional logistic regression models were used to estimate the risk for fertility treatments, which were subclassified into assisted reproductive technology (including *in vitro* fertilization, intracytoplasmic sperm injection and frozen embryo transfer), intrauterine insemination and ovulation induction. We adjusted for maternal age, year of delivery, parity and smoking.
Main results and the role of chance: We found a significantly increased risk for overall fertility treatments in survivors compared to siblings (OR 1.84, 95% CI 1.18–2.86, $P = 0.007$). The risk for fertility treatments was highest among survivors who received radiotherapy (OR 2.24, 95% CI 1.01–4.96, $P = 0.015$). Elapsed time from cancer treatment played a central role, increasing the risk for fertility treatments over time, reaching its peak (OR 2.29, 95% CI 1.17–4.52, $P = 0.003$) 11–34 years from cancer treatments. When it came to age at cancer diagnosis, survivors diagnosed as adults (age 25–34 years) had the highest risk for fertility treatments compared to siblings aged 25 years or older when giving birth (OR 2.31, 95% CI 1.01–5.32, $P = 0.016$).
Limitations, reasons for caution: This study measures risk of fertility treatments among survivors and siblings found in the birth register, leaving out treatments not leading to a live- or stillbirth. Not all fertility treatments are documented in the Finnish birth register. The true incidence of fertility treatments may be higher than in our study.
Wider implications of the findings: Our study supports findings showing that cancer survivors have an increased risk for infertility, with radiotherapy being

the biggest risk when it comes to cancer treatments. Time elapsed from cancer treatment increased the risk for fertility treatments compared to siblings indicating that cancer therapies might lead to diminished ovarian reserves.
Trial registration number: National Institute for Health and Welfare (Dnro THL/1/5.05.00/2014).

O-125 Age at menarche, age at menopause, reproductive lifespan and risk of cardiovascular disease (CVD): a cohort study of 256,284 women

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Study question: Is a longer reproductive period, an indicator of longer endogenous oestrogen exposure, associated with lower incidence of cardiovascular disease (CVD)?
Summary answer: Longer reproductive lifespan is associated with lower incidence of CVD events (fatal and non fatal).
What is known already: Previous studies suggest that endogenous oestrogen has an atheroprotective effect. Early and late menarche and early menopause have been linked with cardiovascular disease, without being clear whether they contribute to greater incidence of new events. A longer reproductive lifespan (defined as age at menopause minus age at menarche) has been found to be associated with a lower estimated 10-year risk of CVD, but whether this translates into a lower incidence of CVD events (fatal and non-fatal) is not known.
Study design, size, duration: The UK Biobank recruited participants aging 40–70 years between 2006 and 2010. 256,284 of White European women were followed up for an average of 5.07 (IQR: 4.38, 5.74) years. 127,949 had gone through natural menopause at baseline, of which 8,686 and 8,768 had been diagnosed with CVD and diabetes respectively.
Participants/materials, setting, methods: Analysis was restricted to those without CVD and diabetes at baseline and with complete data on exposures, outcomes and covariables. Cox regression with adjustment for confounders (age, body mass index, physical activity, smoking, social deprivation index) was used to estimate hazard ratio (HR) of CVD events (fatal and non fatal). Age at menopause and age at menarche were categorical variables, duration of reproductive lifespan was a continuous variable centered for its mean value.
Main results and the role of chance: During 1,252,915 person-years of follow up, 672 women without CVD at baseline developed or died from CVD. The adjusted HR for early menarche (<11 years) or late menarche (>15 years) for new CVD events were 1.14 (95% CI: 0.78, 1.66) and 1.31 (95% CI: 0.93, 1.84) referent to average menarche at the age of 13 years. The adjusted HR for early menopause (<40 years) for new CVD events was 1.74 (95% CI: 1.00, 3.06) referent to average menopause at the age of 50–51 years. The adjusted HR per 1 year longer reproductive period (referent to average duration of 36.7 ± 5.4 years) was 0.98 (95% CI: 0.96, 0.99).
Limitations, reasons for caution: Age of menarche and menopause are self-recorded. 49.9% of the women were still menstruating at baseline and the follow-up period is relatively short.
Wider implications of the findings: Our findings support the hypothesis that a longer reproductive lifespan is associated with reduced risk of incident CVD in a contemporary population of middle aged white women who have experienced a natural menopause.
Trial registration number: N/A.

O-126 Do infertile immigrants have different fertility quality of life and socio-demographic parameters when assisted reproductive technologies (ART) is funded for all?

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Study question: Is fertility quality of life and socio-demographic characteristics different between infertile immigrants and infertile Canadian citizens attending a governmental funded fertility program?
Summary answer: Despite equal funding of ART, immigrants have lower fertility quality of life implying that removing cost does not fully remove the challenges these patients face
What is known already: Governmental funding for ART, including *in vitro* fertilization (IVF), makes it possible for all infertility patients to access expensive treatments regardless of socio-economic status and provides broader access to a more diverse patient population. Nonetheless, some studies have shown that even when funding is available, cultural and ethnic barriers to fertility treatment persist. Infertile immigrants may face additional stresses due to lack of awareness of available services, language barriers, poor social support and significant cross-cultural differences in views concerning infertility. It is of interest to assess fertility quality of life (QoL) in this population when affordability is removed from the equation.
Study design, size, duration: Questionnaires (English or French) were distributed to patients attending a single academic reproductive center between February and August 2015. At this time governmental funding was provided to all residents to cover 3 fresh IVF cycles and all available frozen embryo transfers, as single embryo transfer was obligatory. All patients, males and females, who attended the clinic, were invited to complete the questionnaires anonymously. Questionnaires included socio-demographic items and the Fertility Quality of Life (FertiQoL) questionnaire.
Participants/materials, setting, methods: Overall, 1,020 patients completed the questionnaires, 601 women and 419 partners. Seven hundred and fifty-two (73.7%) were Canadian citizens and 215 (21%) were resident immigrants or refugees. Mean duration of years in Canada in the immigrant group was 4.15 ± 2.9 years. Comparison between study groups was performed by *t*-test and Chi-square test where applicable.
Main results and the role of chance: Canadians were slightly older (36.4 ± 5.9 vs. 35.1 ± 4.8, $p = 0.002$) with higher rates of secondary infertility compared to immigrant patients (34 vs. 21.8%, $p = 0.001$). Previous IVF attempts and Infertility duration were similar (1.94 vs. 1.74 previous attempts, $p = 0.1$; 2.51 vs. 2.56 years of infertility, $p = 0.6$). While immigrant patients were more likely than their Canadian counterparts to have university or graduate degrees (74.8 vs. 63.5%, $p = 0.004$), they were also more likely to be unemployed and have lower annual household incomes (35.8 vs. 13% unemployed, $p < 0.05$; 62.8 vs. 32.9% income <65,000$, $p < 0.05$,). Immigrants reported poorer QoL than their counterparts: they experienced their infertility with distressing emotions, negative thinking, more physical symptomatology, less social support and experienced clinic environment as more problematic. This is reflected by their lower FertiQoL scores in the corresponding domains: emotional (57.9 vs. 62.2, $p = 0.02$), mind/body (63 vs. 67.4, $p = 0.02$), social (67.3 vs. 71, $p = 0.03$) and environment (67.4 vs. 70.7, $p = 0.03$). Total Core and treatment scores were also lower for immigrants (64.7 vs. 68.1, $p = 0.02$; 65.6 vs. 69.2 $p < 0.001$, respectively), meaning poorer fertility QoL. Scores in the relational and tolerability domains were also higher for Canadian although differences were not statistically significant (70.6 vs. 72.1, $p = 0.3$; 64 vs. 67.1, $p = 0.1$, respectively).
Limitations, reasons for caution: Not all patients agreed to participate in the survey and fill out the questionnaires. Some immigrant patients may have declined participation due to lack of knowledge in both English and French.
Wider implications of the findings: This is the first study indicating cost is not the only factor affecting how patients experience infertility. Ethnic and cultural factors may affect how immigrants cope with infertility. This population may be at greater risk for depression and anxiety (co-morbidity with QoL) and should be flagged for possible psychosocial intervention
Trial registration number: None.

O-127 Predicting the chances of having a baby after one or more complete cycles of *in vitro* fertilisation: analysis of linked cycle data from 113,873 women

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Study question: What are the predicted cumulative chances of a live birth following multiple complete cycles of *in vitro* fertilisation (IVF) for couples with different characteristics?

Summary answer: The chance of a live birth for a 30-year-old woman with 2 years unexplained infertility is 80.1% after three IVF cycles (93.3% after six).
What is known already: The predicted chance of a live birth following IVF has traditionally been reported after a single fresh cycle. However, many couples are unsuccessful after their first embryo transfer and wish to know their chances of having a baby after multiple complete cycles – each involving the transfer of fresh as well as frozen-thawed embryos. The ability to estimate the cumulative probability of live birth per woman based on pre-and post-treatment characteristics would enable more informed decisions at different time points tailored to the couple's individual situation.
Study design, size, duration: In this retrospective cohort study, linked cycle data of 113,873 women who had their first IVF treatment attempt in the UK between January 1999 and September 2008 were included. Every complete cycle, defined as all fresh and frozen embryo transfers resulting from one episode of ovarian stimulation, were linked to individual women. All complete cycles were included up to the end of treatment exposure (September 2009) or first live birth occurrence, whichever came first.
Participants/materials, setting, methods: A database containing all UK IVF treatments was obtained from the Human Fertilisation and Embryology Authority (HFEA). Using a logistic regression model, the predicted probability of live birth over six complete cycles of IVF was calculated pre-treatment using couple characteristics and after the first fresh embryo transfer (post-treatment) using both couple and treatment characteristics. The model was assessed for predictive ability using the C-statistic to measure discrimination and calibration plots.
Main results and the role of chance: After exclusions, 113,873 women underwent 184,269 complete cycles. A total of 33,154 (29.1%) women had a live birth in the first complete cycle. In order of importance, the key predictors in the pre-treatment model were female age [odds ratio 0.60, 95% confidence interval (0.58–0.62), (change from 31 to 37 based on interquartile)] and duration of infertility (years) [0.97 (0.97–0.97)]. In the post-treatment model they were number of eggs collected [1.32 (1.29–1.35), (change from 5 to 13 eggs)], cryopreservation of embryos (yes vs. no) [1.95 (1.90–2.00)], female age [0.64 (0.62–0.66)] and stage of embryos transferred [e.g., double blastocyst vs. double cleavage, 1.83 (1.71–1.96)]. The *C*-statistics were 0.635 and 0.750 (cycle 1) for pre- and post-treatment models respectively. Calibration was good. In a couple with 2 years of primary unexplained infertility embarking on IVF, where the female partner is 30 years old, the chance of a baby after three complete cycles is 80.1%. At the point of transfer of a blastocyst in the first fresh cycle in which 15 eggs were collected and any spare embryos cryopreserved, the chance of a live birth updates to 95.8% after three complete cycles. In a woman aged 40 these chances are 39.9 and 74.3% respectively.
Limitations, reasons for caution: Patients who discontinued treatment were censored in the analysis meaning that their future chances of a live birth were assumed to be similar to those who continued which may lead to over-optimistic estimates of predictions. The model has yet to be externally validated in another national IVF registry.
Wider implications of the findings: These clinical prediction models can be used to counsel couples who want to know their overall chances of having a baby over the complete IVF journey. The results may be used by policy-makers to determine the provision of an optimum number of IVF cycles per couple based on their characteristics.
Trial registration number: N/A.

O-128 Poor financial recovery from out-of-pocket payment for assisted reproductive technology (ART) in the public-academic health sector of South Africa

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Study question: How do households (HHs) recover financially from out-of-pocket payment (OOP) for assisted reproductive technology (ART)?
Summary answer: After a minimum of 2.5 years of follow up, the large majority of HHs (86.3%) had not fully recovered financially.
What is known already: OOP for health is wide spread in developing countries. It is the most inequitable way of financing health care. A previous study involving 135 couples undergoing ART with conventional ovarian stimulation at a level 3 referral institution in the public-academic health sector of South Africa documented that OPP created catastrophic expenditure in 20% of participating HHs. HHs engaged in a range of financial coping strategies, often concomitantly, including accessing savings, borrowing money, selling assets, reducing household expenditure and undertaking additional work.
Study design, size, duration: An observational follow up study was conducted in order to assess the financial recovery of HHs from OOP for ART. Seventy three participants were interviewed 2.5 years or more after the original OOP. Data collection occurred between November 2014 and June 2015
Participants/materials, setting, methods: All contactable informants from the original study were invited to participate. A questionnaire capturing information on socioeconomic status, new financial demands, and financial recovery from the previous OOP for ART was administered. Most interviews were conducted telephonically. Markers of lack of financial recovery included outstanding loans, non-recovery of sold assets or savings, ongoing performance of additional work to supplement income and ongoing reduction of HH expenditure.
Main results and the role of chance: The follow up participation rate was 51%. Only 10 of the 73 HHs (13.7%) reported full recovery, defined as all loans repaid, assets/savings regained and no ongoing reduction in HHs expenditure or performance of additional work. HHs in the highest socioeconomic tertile showed better full or partial recovery when compared to those in the lowest. Forty HHs reported new financial demands because of a baby or other HH events, however no association was found between this and lack of financial recovery. Specific coping strategies utilised and lack of financial recovery were as follows: (1) 35 HHs had taken out loans and 14 (40%) of these were still in debt; (2) 68 HHs had accessed savings which 45 (66.2%) had not regained; (3) 13 HHs had sold assets which 10 had failed to re-purchase (77%); (4) 39 HHs took on additional work which 20 (51.3%) were still performing; (5) 68 HHs had reduced HH expenditure and 51 (75%) continued to do so. The role of chance is minimal.
Limitations, reasons for caution: The number of participants was small and results were dependant on informant recall. Associations shown cannot be interpreted as causality.
Wider implications of the findings: This is the first study assessing financial recovery after OOP for ART. While the results apply to the study group and setting, it is possible that absence of financial risk protection for ART is associated with poor financial recovery from OOP in other settings, especially in low income countries.
Trial registration number: Not applicable.

SELECTED ORAL COMMUNICATIONS

SESSION 35: AMH: THE PLOT THICKENS

Tuesday 05 July 2016 **Room 101** **10:00–11:30**

O-129 The bone morphogenetic protein 15 up-regulates the anti-Müllerian hormone receptor expression in granulosa cells

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Study question: The study aimed to investigate the regulation of the anti-Müllerian hormone (AMH) receptor gene (*AMHR2)* expression by bone morphogenetic proteins (BMPs) in granulosa cells (GCs).
Summary answer: BMP15 stimulates *AMHR2* expression in GCs from different maturational stages follicles both *in vitro* and *in vivo*.
What is known already: Anti-Müllerian hormone (AMH) is produced by the GCs of growing follicles and inhibits follicular development. BMPs are important regulators of folliculogenesis. Ewes with loss of function mutations in the

oocyte-derived *BMP15* or *GDF9* genes are sterile when the mutation is present at the homozygous stage, but heterozygote ewes are unexpectedly hyperprolific. Recently, several BMPs including BMP15 and the granulosa/theca-derived BMP4 have been shown to enhance *AMH* expression in GCs, but their effect on *AMHR2* expression is unknown.

Study design, size, duration: The effects of BMP15, GDF9 and BMP4 on *AMHR2* expression were studied in human GCs (hGCs) from women undergoing assisted reproduction technology procedures and in ovine GCs (oGCs) from small antral follicles. The effects of BMPs on human *AMHR2* promoter activity were analyzed in oGCs transfected with a luciferase reporter construct. The *in vivo* effect of BMP15 on *AMHR2* expression in GCs was investigated on ewes with genetically impaired BMP15 biological activity.

Participants/materials, setting, methods: mRNA accumulation of *AMHR2*, *AMH* and other BMP target genes was quantified by real-time RT-PCR. The activity of the human *AMHR2* and *AMH* promoter reporter constructs (2,252 and 423 base pairs respectively) was quantified by the measurement of their luciferase activity.

Main results and the role of chance: BMP15 and BMP4 significantly enhanced *AMHR2* mRNA levels in hGCs (1.8-fold, $p < 0.001$ and 2-fold, $p < 0.001$, respectively) and in oGCs (1.4-fold increase, $p < 0.05$ and 1.5-fold increase, $p < 0.001$, respectively) whereas GDF9 acting alone or in combination with BMP15 had no effect. In oGCs, BMP15 and BMP4 enhanced also *AMH* mRNA levels, and GDF9 increased the stimulating effect of BMP15. In keeping with these results, BMP15 and BMP4, but not GDF9, enhanced *AMHR2* promoter activity in oGCs (1.2-fold increase, $p < 0.01$ and 1.3-fold increase $p < 0.05$, respectively), whereas GDF9 increased the stimulating effect of BMP15 on *AMH* promoter activity. Moreover, oCGs from both Lacaune and Rasa Aragonesa ewes, carrying loss-of-function mutations in *BMP15*, had reduced *AMHR2* mRNA levels compared to wild-type animals (6.6-fold decrease, $p < 0.01$ and 3.4-fold decrease, $p < 0.001$, respectively). Interestingly, these mutations had no effect on *AMH* expression. Altogether, these results suggest that the mechanisms of action of BMP15 on *AMHR2* and *AMH* expression are different.

Limitations, reasons for caution: The hCGs were luteinized, but they still express both *AMH* and *AMHR2*. Transfection studies were only performed in oCGs because hCGs are very difficult to transfect.

Wider implications of the findings: Altogether, these results suggest that BMP15 stimulate *AMHR2* expression in GCs of different maturational stages, thereby enhancing AMH actions in the follicular microenvironment. Hyperprolificity in ewes could thus be due to a decrease in both BMP and AMH inhibitory actions at the FSH-dependent phase of terminal follicular development.

Trial registration number: 0

O-130 Comparable inter-laboratory performance of two automated platforms (Roche® and Beckman Coulter®) for Anti-Müllerian Hormone (AMH) evaluation permits greater clinical confidence when assessing functional ovarian reserve

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Study question: To use multi-centre objective data to compare the performance characteristics of the automated AMH assays from Roche® (Elecsys system) and Beckman Coulter® (Access2 system).

Summary answer: Absolute concentrations showed strong agreement and correlation ($R^2 = 0.971$) between the systems. Inter-laboratory variance was identical, and low, for both assays (less than 5.5%).

What is known already: AMH can be used to predict ovarian responses to stimulation, allowing individualization of stimulation protocols, FSH doses, and prediction of IVF outcome. However, the original manual enzyme-linked immunosorbent assays (ELISA) were prone to methodological errors due to numerous factors, not least the requirement for skilled operators in many laboratories where (semi)-automation was not performed. Consequently, high inter-laboratory variability was observed and confidence in the assay was adversely affected. Two automated assays are now available which use the same antibodies, but deploy different technologies and calibration. The comparability of these assays needs to be established for clinical use.

Study design, size, duration: Objective multicentre comparative cohort study. The reported AMH results from 40 serum samples (distributed in 8 batches) were sent to contributing laboratories running either the Access2 or the Elecsys system from April to November 2015 (inclusive) as part of the UK national quality assurance (NEQAS) accreditation scheme, and were analysed by the NEQAS team prior to reporting by one of the contributing laboratories.

Participants/materials, setting, methods: The absolute concentrations and the inter-laboratory variance were assessed and compared for sample AMH values from the 40 samples analysed in eight batches for the two assay platforms. A total of 1,279 individual results were reported and assessed per assay.

Main results and the role of chance: The reported median concentrations ranged from 0.9 to 30 pmol/L, and there was a close agreement between the two assay methods. The absolute concentrations were determined as: Elecsys = 0.973 × Access2 + 0.69. The Pearson's correlation evaluation was high (R^2 value = 0.971) which demonstrated a high degree of agreement and precision between the two automated assays.

The variance between laboratories was low throughout the series (<5.5% for both assay methods) and there was no difference between them.

Limitations, reasons for caution: These objective multicenter data are strong evidence that the two automated assay systems report very similar absolute values and that although they use different technologies and calibration, the results are equally reliable and, importantly, reproducible in different laboratories. They do not identify how results relate to other assays.

Wider implications of the findings: Both the Roche® (Elecsys) and Beckman Coulter® (Access2) platforms give consistent, reproducible and comparable AMH results. This should minimize the inter- and intra-laboratory variations previously identified, and result in a return of clinicians' confidence in the method of assessing ovarian reserve and assigning treatment protocols based on these results.

Trial registration number: N/A.

O-131 Unraveling the long-term decline of anti-Müllerian hormone

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[3]*National Institute for Public Health and the Environment, Centre for Nutrition, Prevention and Health Services, Bilthoven, Netherlands*

Study question: Are long-term serial anti-Müllerian hormone (AMH) measurements consistent in their decline pattern between and within individuals?

Summary answer: In a longitudinal cohort of 3,326 women spanning 20 years, AMH declined in similar fashion between individuals and age-dependent levels remained stable within individuals.

What is known already: AMH is a marker of ovarian reserve and is suggested to aid in the prediction of time to menopause. Although AMH levels are widely known to decrease with age, there is still a lack of information regarding longitudinal AMH decline over a longer period of time.

Study design, size, duration: Our study population consisted of women enrolled in the longitudinal population-based Doetinchem Cohort Study. We included 3,326 women who completed at least one of five consecutive follow-up rounds between 1987 and 2010, leading to a maximum follow-up time of 20 years. At each follow-up round, data collection included a questionnaire, anthropometric measurements and blood withdrawal.

Participants/materials, setting, methods: AMH was measured in 12,929 serum samples with the picoAMH assay (AnshLabs). A latent class analysis was used to determine whether there were distinct decline patterns between individuals. The correlation of age-dependent AMH levels within individuals was studied using a linear mixed model with smoothing splines for age. The variation of the distance of each measurement to the group average at that age was compared to the between-individual variation, resulting in an intra-class correlation coefficient.

Main results and the role of chance: At baseline, mean age was 39 ± 10 years, median AMH [IQR] was 1.00 [0.04–3.07] ng/mL and 86% of the women was premenopausal. The mean number of follow-up rounds per woman was 3.9. Latent class analysis revealed that women could be grouped into one of two distinct decline trajectories with a similar and parallel shape. In both groups, AMH levels decreased slowly until the age of 40 and quickly thereafter, reaching the detection limit (0.002 ng/mL) a few years before and after the age of 50, respectively. After adjustment for confounders, the distances of serial AMH

measurements to the age-specific group average were highly correlated within individuals, with an intra-class correlation coefficient of 0.87.
Limitations, reasons for caution: Absolute measured AMH levels may differ per type of AMH assay, but this is not expected to affect decline trajectories measured with a single assay.
Wider implications of the findings: This is the first large-scale study to measure longitudinal AMH decline over a long period of time. These data will provide further insight into the value of AMH in a clinical setting.
Trial registration number: N/A.

O-132 Anti-Müllerian hormone levels and features of polycystic ovary syndrome in adolescence

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Study question: Can serum AMH levels at age 15 predict features of polycystic ovary in early adulthood (at 18 years)?
Summary answer: AMH is related to features of PCOS in adolescence.
What is known already: Irregular menstrual cycles during puberty, especially oligo- or secondary amenorrhoea, have been considered to be physiologic. However, frequently oligo- or secondary amenorrhoea is not transient. A considerable part of oligomenorrhoeic adolescents have clinical, endocrine and ultrasound signs of PCOS. Defining appropriate diagnostic criteria for diagnosis of PCOS in adolescence is a relevant challenge. AMH levels are elevated in adult women with PCOS, and therefore increasingly used as a diagnostic feature. The use of adolescent serum AMH levels as reliable predictor of persistent oligo- or secondary amenorrhoea, as part of PCOS, can be of great value in (young) adult life.
Study design, size, duration: Subjects are part of a large, prospective, cross-sectional population-based, observational study that aimed to collect comprehensive data on the endocrinology of menstrual cycle disorders in a general population performed between 1990 and 1993 (van Hooff[4] M, Hum Reprod, 2004). We identified 272 adolescents who underwent blood sampling. In 160 individuals, ultrasound data of the ovaries were also available.
Participants/materials, setting, methods: Participants were categorized at age 15 by menstrual cycle pattern: regular cycle (n = 112), irregular cycle (n = 86), oligomenorrhoea & secondary amenorrhoea (n = 61), primary amenorrhoea (n = 4) and polymenorrhoea (n = 9). The relation between AMH (levels and menstrual cycle pattern, physical examination, hormonal measurements and ultrasound pattern of the ovaries was determined. The predictive value of AMH (age 15) for oligo-amenorrhoea at age 18 was made by a ROC-analysis.
Main results and the role of chance: The mean (±SD) AMH levels (at age 15) in the regular, irregular and oligo-amenorrhoeic group were 3.2 (2.3) µg/L, 3.9 (2.6) µg/L and 5.1 (3.6) µg/L, respectively. The AMH level was significantly higher in the oligo- or secondary amenorrhoea group compared to the regular cycle group ($p < 0.01$).
AMH was positively correlated with signs of hirsutism ($r = 0.20$, $p < 0.01$). AMH was positively correlated with the number of the follicles in the ovaries determined by ultrasound ($r = 0.28$, $p < 0.05$): in both ovaries <5 follicles: AMH 3.2 µg/L; one ovary 5–9 follicles, one ovary <5 follicles: AMH 3.6 µg/L; both ovaries 5–9 follicles: AMH 4.1 µg/L; both ovaries ≥10 follicles: AMH 6.3 µg/L ($p < 0.01$).

An AMH cut-off level of 6.01 µg/L at the age of 15.3 year had a sensitivity of 48.6% and specificity of 85.2% for predicting oligo- or secondary amenorrhoea after 3 years. An AMH cut-off level of 7.99 µg/L at the age of 15.3 year had a sensitivity of 29.7% and specificity of 94.4% to predict oligo- or secondary amenorrhoea after 3 years.
Limitations, reasons for caution: Limitations of our study are that the serum samples were taken in the nineties, and that not all participants could be followed-up after 3 years. The predictive value of AMH with regard to PCOS at older age needs to be established.
Wider implications of the findings: This study contributes to the improvement of prediction on developing PCOS in adolescence and the use of AMH in this diagnostic challenge. Improvement of prediction of PCOS can generate opportunities for starting early secondary prevention and intervention of metabolic and cardiovascular health aspects with regard to PCOS.
Trial registration number: None.

O-133 Phenotypic variation in anti-Mullerian hormone (AMH) production per follicle in women with polycystic ovary syndrome (PCOS) and isolated polycystic ovarian morphology (PCOM)

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Study question: Is there phenotypic variation in the anti-Mullerian hormone production per follicle in polycystic ovary syndrome and what are the parameters associated with this variation?
Summary answer: Anovulatory phenotypes of PCOS have a higher per follicle AMH production as compared to ovulatory phenotypes and PCOM due to intrinsic granulosa cell dysfunction
What is known already: Women with polycystic ovary syndrome (PCOS) have higher levels of anti-Mullerian hormone (AMH) than non-PCOS women. This is due to higher numbers of antral follicles and a greater production of AMH per follicle. AMH plays an inhibitory role in folliculogenesis, reducing the sensitivity of growing follicles to FSH. AMH may have a key role in the pathogenesis of anovulation in PCOS.
Study design, size, duration: The study was a prospective cross-sectional study with 218 participants undergoing evaluation for subfertility from January to December 2015. The study included women with PCOS who met the Rotterdam criteria (n = 156) and women with isolated PCOM (n = 62). Women with PCOS were classified into four phenotypes A, B, C and D based on the inclusion criteria of oligo-anovulation (OA), hyperandrogenism (HA) and polycystic ovarian morphology (PCOM).
Participants/materials, setting, methods: Participants undergoing evaluation for subfertility at the fertility clinic were screened for eligibility. Routinely collected data for age, BMI, waist-hip ratio, modified Ferriman–Gallway score, FSH, LH, testosterone, AMH, AFC and insulin were collated. A ratio of serum AMH to AFC was used as a surrogate marker for per-follicle AMH production. The ratio and above parameters were compared in the four phenotypes of PCOS and isolated PCOM. Data was analysed using the IBM SPSS software, v22.0.
Main results and the role of chance: The median AMH/AFC ratios in the groups were: Group A (OA + HA + PCOM) = 1.44, Group B (OA + HA) = 0.97, Group C (HA + PCOM) = 1.10, Group D (OA + PCOM) = 1.70 and PCOM = 1.20. A significant difference was seen in the AMH/AFC ratio amongst the different phenotypes of PCOS and PCOM ($F = 4.457$, $p = 0.005$). Phenotype B had very small numbers and was excluded from analysis. PCOS oligo-anovulatory phenotypes (group A and D) had a significantly higher per follicle AMH than PCOM ($p = 0.048$ and $p = 0.028$ respectively) and a borderline significant difference with phenotype C ($p = 0.057$). There was no significant difference between the ovulatory PCOS phenotype C and PCOM ($p = 0.999$). (One-way analysis of variance, post-hoc Tukey).

Age, BMI, waist-hip ratio, modified Ferriman-Gallway score, FSH, LH, testosterone and insulin were not significantly different in the four PCOS phenotypes compared. Only testosterone was significantly higher in phenotype group C as per definition as compared to PCOM ($p = 0.017$).

The correlation between testosterone and the AMH/AFC ratio was however found to be non-significant ($r = 0.012$, $p = 0.30$).
Limitations, reasons for caution: The operator variability in AFC measurement and the contribution of follicles less than 2 mm to the AMH may affect the AMH/AFC ratio. The study included women attending the fertility clinic and did not include women with extremes of BMI. The results are hence relevant only to the above population.
Wider implications of the findings: A higher per-follicle AMH production in anovulatory phenotypes of PCOS indicates a key role of AMH in the pathophysiology of anovulation associated with AMH. Lack of correlation of testosterone with AMH/AFC ratio and the highest ratio in phenotype-C of PCOS point to an intrinsic granulosa cell dysfunction leading to anovulation.
Trial registration number: Trial registration not required as observational study.
Local R&D approvals in place.
No ethical approval needed.

O-134 Irrespective of age, serum anti-Müllerian hormone (AMH) levels are related to miscarriage rates in IVF-ET thereby supporting the hypothesis that AMH is a qualitative biomarker

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Study question: Are serum AMH levels independently related to miscarriage rates after IVF-ET?
Summary answer: Irrespective of age, patients with low AMH levels display higher miscarriage rates after IVF-ET. This strongly suggests that AMH is a biomarker of oocyte quality.
What is known already: Whereas, by some authors, AMH has been recognized as an age-independent marker of pregnancy rates after IVF-ET, it remains unclear whether this relationship would be a mere result of increased oocyte yield or a consequence of improved oocyte competence. A methodological strategy to gain insights into this issue would be to focus not only on pregnancy rates but also on the analysis of miscarriage rates in age-controlled patients showing different AMH levels. Indeed, miscarriages often result from an abnormal chromosomal status of the embryo, thereby being an additional marker of oocyte-embryo health.
Study design, size, duration: We studied 2,493 non-PCOS patients (AMH levels <7.10 ng/mL) who have had a reference, centralized serum AMH determination within 12 months before IVF-ET. Of them, 2,052 were sorted into two opposite age groups: 34 years (Young group; $n = 1,114$) and 37 years (Old group; $n = 938$). In addition, we determined three AMH groups according to extreme quartile values: low AMH (0.10–1.60 ng/mL; $n = 475$), intermediate AMH (1.61–3.99 ng/mL; $n = 1,046$), and high AMH (4.00–7.10 ng/mL; $n = 531$).
Participants/materials, setting, methods: All patients received conventional controlled ovarian stimulation (COH) for IVF-ET. In the extreme age population (Young and Old groups; $n = 2,052$), the relationship between AMH groups and IVF-ET outcome (clinical pregnancy, miscarriage, and live birth rates) was assessed by Chi-square test. The Kruskall–Wallis test was used when appropriate. In whole population ($n = 2,493$), binary logistic regression was conducted to verify whether predictability of AMH was independent of age.
Main results and the role of chance: As expected, AMH was associated to the intensity of the ovarian response to COH in both age groups. In the Young group, we observed a stepwise increase in clinical pregnancy (26.2, 43.2, and 47.2%; $P < 0.001$) and live birth (19.1, 34.6, and 40.2%; $P < 0.001$) rates across the low, intermediate, and high AMH groups, respectively. Similar figures were observed in the Old group: 21.6, 32.4, and 36.7% ($P < 0.001$) and 14.4, 22.1, and 28.5% ($P < 0.001$). Further, miscarriage rates decreased stepwisely from the low, intermediate, high AMH groups, respectively, in the Young group (22.9, 16.8, and 11.0%) and in the Old group (31.1, 26.2, and 20.7%). Differences of miscarriage rates reached statistical signification only between the low and the high AMH groups in the Young group ($P < 0.05$), probably due to limitations of sample size. Yet, binary logistic regression run over the whole population confirmed the association between serum AMH and miscarriage rates (together with clinical pregnancy and live birth rates) and this independently of age.
Limitations, reasons for caution: Unfortunately, preimplantation aneuploidy screening of embryo in low, intermediate, and high AMH patients was not available in the present investigation. Larger series of clinical pregnancies in older women are needed to confirm the relationship of AMH and miscarriage rates in this subset of patients.
Wider implications of the findings: The independent relationship between AMH and pregnancy outcome suggests that AMH is not only a quantitative but also a qualitative biomarker of oocyte-embryo competence. Young and old patients should be aware of the added value of this marker over age itself for reaching a live birth after IVF-ET.
Trial registration number: Not Applicable.

INVITED SESSION

SESSION 36: EUROPEAN AND GLOBAL ART MONITORING SESSION

Tuesday 05 July 2016 **Hall 5 A** **11:45–12:45**

O-135 Assisted reproductive technology (ART) in Europe 2013. Preliminary results generated from European registers by ESHRE

O-136 ICMART World Report 2012

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Abstract text
Study question: In 2012, what was: global utilization of ART as measured by number and type of cycles; effectiveness as measured by clinical pregnancy, miscarriage and live birth rates; safety as measured by multiple births and other complications; and the status of extensive additions and revisions to the ICMART/WHO glossary?
Summary answer: Globally, ART utilization continues to increase but with wide variation in utilization, effectiveness and safety. Participation in data collection is gradually improving. The greatly expanded ICMART/WHO glossary is essential for standardization of concepts, processes, procedures, clinical events and research that is essential for precise communication in ART.
What is known already? ICMART began ART global data collection in 1991. Utilization, effectiveness and safety have continuously improved with more cycles, higher pregnancy rates and lower rates of multiple births, respectively. Wide variations exist globally. Over 6 million ART babies have been born. China does not report. ICMART is helping develop African registries. Data collection and quality remain challenging. The ICMART/WHO glossary has undergone major revisions and expansion. This process is complex and culturally sensitive.
STUDY design, size, duration: Countries and regions annually collect ART data, some prospectively and others retrospectively. ICMART retrospectively requested these data from all known global sources for 2012, analyzed them and presents preliminary results. The ICMART/WHO glossary update involves dozens of reproductive specialists over 3 years in an interactive consensus process.
Participants/materials, setting, methods: The European IVF Monitoring Consortium (EIM), SART, Latin American Network of Assisted Reproduction (REDLARA), Australian/New Zealand Registry, and other countries, totaling approximately 60, contributed national data through standard ICMART forms. Data were reviewed, corrected, analyzed and summarized by ICMART working with the University of Uppsala, Sweden using standard statistical tests.
Main results and the role of chance: Data collection and analysis are ongoing. Preliminary results only will be presented at ESHRE. The ICMART/WHO Glossary has been expanded to include 5 sections: clinical terminologies, outcome terminologies, epidemiology and public health, laboratory including andrology and embryology.

Most previous definitions have been retained but some modified, clarified or expanded. Many new definitions have been added.
The role of chance is minimal.
Limitations, reasons for caution: Global results are limited to reporting countries and clinics, approximately 2/3 of global cycles. Many countries have limited data validation and ICMART can perform only minimal data validation. The ICMART/WHO glossary definitions are based on a consensus process by many experts covering different disciplines, and although difficult, the work ends when universal agreement is reached.
Wider implications of the findings: It is accepted that measurement of human activities improves quality and outcomes. ICMART World Reports standardize data, track trends, enable comparisons, stimulate questions and improve ART quality in clinics, countries, regions and globally. Additionally, better understanding of ART increases societal acceptance and eventually creates broader support for ART research and clinical access. The ICMART/WHO glossary provides a common terminology platform to increase quality of ART research and clinical data and communication.
Study funding: American Society for Reproductive Medicine, European Society for Human Reproduction and Embryology, Society of Assisted Reproductive Technology, Japan Society for Reproductive Medicine,

Japan Society of Fertilization and Implantation, Latin American Network of Assisted Reproduction, Fertility Society of Australia, Government of Canada, Ferring Pharmaceuticals.

O-137 ICMART in Africa

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Abstract text
The need to document utilization, effectiveness and safety of ART is established and met through national and regional data registries in most parts of the world. The International Committee Monitoring Assisted Reproductive Technologies (ICMART) generates annual reports on global data and assists countries and regions in establishing ART registries. There is a need for registry development in sub-Saharan Africa as currently only one national registry exists. ICMART has initiated and facilitated communication among role players in sub-Saharan Africa regarding the formation of a regional ART registry. A project plan has been generated modeled on the Latin American Registry for ART. The Latin American registry software, which captures case-by-case data, has been made freely available to ART centres in sub-Saharan Africa and is currently being piloted. Training materials and standard operating procedures for data collection are being developed. The clinical and laboratory directors of all ART centres in the region will be invited to participate in the registry and to submit data to the regional registry hub. The anonymity of centres and patients will be protected. The registry hub will report back (1) to each centre its own data confidentially; (2) to each country the pooled national data; and (3) to the region the pooled regional data pertaining to utilization, effectiveness and safety of ART. A regional meeting of participants is in planning.

The current absence of data pertaining to utilization, effectiveness and safety of ART in sub-Saharan Africa compromises the evidence-base for ART in the region. This has implications for health care providers, users and planners and other stakeholders. Registry data, once generated, will provide a platform for clinical and health political improvement strategies and for research as well as national and regional benchmarks against which trends and improvements can be monitored.

INVITED SESSION

SESSION 37: UPDATE ON ULTRASOUND IMAGING

Tuesday 05 July 2016 **Hall 3 AB** **11:45–12:45**

O-138 3D ultrasound to diagnose uterine anomalies

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Abstract text
After the introduction of ESHRE-ESGE classification of uterine anomalies (2013) a lot of arcuate uteri classified according to ASRM/Salim classification (2003) became partial septate uteri (class U2a). Furthermore also dysmorphic T shaped uteri are difficult to assess in all classification, also the new ESHRE/ESGE classification, class dysmorphic uteri (U1c), for absence of precise diagnostic criteria.

We propose new criteria for the detection of T shaped by means of angle between isthmic cavity and fundal endometrial rim layer and fundal/isthmic cavity widths. We also re-evaluated and reclassified according ESHRE/ESGE classification, 362 stored 3D ultrasound uterine volume of women with previous diagnosis of arcuate uterus.

The assessment of uterine morphology was performed in a 3D coronal plane and following measurements were taken: (1) fundal cavity width (W1) (the distance between the two internal tubal ostia), (2) width of uterine cavity at corpus-isthmic level (W2), (3) uterine fundal wall thickness (M) (the distance from interostial line and the external uterine serosa), (4) the lateral angle between the corpus-isthmic cavity and the two fundal endometrial layers (A right, A left), (5) in case of cavity fundal indentation, the indentation length (L) (the distance from the tip of the fundal indentation to the interostial line) and fundal indentation angle (α) (the angle between the two endometrial layers). Recorded reproductive history of the patients was correlated to the measurements and to normal and subseptate uterus according ESHRE/ESGE classification.

Of the 362 previous arcuate uteri, 189 (52.2%) become septate according to ESHRE classification because the L exceeded the 50% of the fundal uterine wall thickness (M) while 173 (47.8%) were considered slightly dysmorphic or normal uteri. Of the reclassified patients with septate uterus, a higher percentage (but not statistically significant) of women with infertility and at least one miscarriage was observed compared to the patients with normal uterus.

T-shaped uterus had a fundic cavity significantly wider than normal uterus, while the width at corpus-isthmic level was statistically lower in T-uterus compared to normal and septate. Furthermore dysmorphic T shaped uteri showed more acute lateral angles that were absent or wide in the two control groups. Compared to normal uteri a higher rate of infertility in patients with T shaped uterus (61.9 vs. 47.9%) was observed. A higher percentage of miscarriage in women with septate uterus compared to T shaped has been shown (45.8 vs. 23.8%).

ESHRE-ESGE classification arise several diagnostic and clinical implications and may result in difficulties in counseling and in treatment options in women with U2a and U1c uteri who experience infertility and/or miscarriage. The width and not the length of the septum seem to have an impact on reproduction. Dysmorphic T-shaped uterus has a typical different morphology than other uterine anomalies, easily reproducible with standardized measurements. Lateral angles and corpus-isthmic ratio measured on 3D coronal section proposed in this study improve the diagnosis of dysmorphic uteri and could define better selection criteria for hysteroscopic treatment, resulting in improved fertility. The new ESHRE/ESGE classification is not supported by retrospective results and prospective studies of corrective surgery are needed that could help to define selection criteria for metroplasty, resulting in improved long-term outcomes.

O-139 3D ultrasound in early pregnancy

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Abstract text
The last decennium there is an increasing interest in Early Pregnancy ultrasound, not only for determining pregnancy localization and viability but also for the detection of abnormal growth and development, including early detection of various congenital anomalies. Safety issues are served by a reduction of the exposure time due to off-line analysis of transvaginal 3D volumes. Although the 3D US technology has improved further, the images are still presented on a 2D screen without offering natural depth perception. A special volume rendering application (V-Scope®) has been developed at the Erasmus Medical Centre in Rotterdam to create virtual "*holograms"* of the 3D US datasets using projection-based 3D displays as well as a smaller and cheaper 3D desktop VR systems. Manipulation (crop, rotate, resize, and clip plane function) of the holograms is natural, intuitive and very easy to learn even for untrained sonographers. This technology allows very precise and accurate length measurements, even of non-linear structures, as well as semi-automated volume measurements.

With this innovative 3D VR approach first trimester reference curves were constructed for CRL, BPD, HC, AC, umbilical cord length, vitelline duct length, cerebellum, diencephalon, mesencephalon, telencephalon and hand length and hand- and wrist width. Furthermore reference values were established for embryonic volume and volumes of the placenta, gestational sac, yolk sac, and brain cavities. These reference curves serve as background information for evaluation of normal embryonic growth and development. Using 3D VR it is even possible to determine the Carnegie Stages of embryonic development. The use of 3D power Doppler volumes in VR also enables the measurement of the total placenta bed vascular volume and the total placenta vascular volume, these being promising parameters for predicting second and third trimester fetal growth restriction.

3D VR appears to be superior in diagnosing first trimester surface abnormalities and skeletal/limb malformations in comparison to 2D and 3D US. In case of anomalies like TAR syndrome, CNS defects and conjoined twins additional abnormalities were observed using 3D VR as compared to 2D and 3D US. In comparison to normal pregnancies embryonic volume measurements turn out to be smaller in aneuploid pregnancies and some types of structural congenital anomalies. In detecting early abnormal growth the measurement of embryonic volume performs better than CRL measurements. However, embryonic volume does not perform better than CRL in predicting miscarriages.

Studies on genetic and environmental influences in the Rotterdam Predict Study (Steegers-Theunissen et al., 2015), using 3D VR, revealed influences of periconceptional use of folic acid, maternal age, dietary patterns, smoking, alcohol and vitamin B12 levels on normal growth and development. Embryonic growth and development are known to be related to birth weight.

These various findings led to the introduction of the new theme *Embryonic Health* with 3D VR as an important and innovative tool for screening, prediction and diagnosis in this period of pregnancy with an increasing interest of both professionals and pregnant women. Documentation of first trimester physiological omphalocele, clubfoot, embryonic curvature and sex determination are now subject of 3D VR studies, as is the analysis of embryonic movement. With the development of the 3D VR desktop system this new 3D VR technology has become available as a new tool for further collaborative studies and clinical use at outpatient clinics. As 3D TV-screens are also becoming more popular it may be expected that in the near future 3D VR will be build in as a standard feature in ultrasound equipment.

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INVITED SESSION

SESSION 38: REPRODUCTIVE ENDOCRINOLOGY/EARLY PREGNANCY

Tuesday 05 July 2016 **Hall 3 DE** **11:45–12:45**

O-140 Monozygotic twinning: to be or not to be monoclonal

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Abstract text

We are polyclonal beings since each person is born of a unique fertilization event.

Rarely however, two babies or more develop from a single embryo, producing monoclonal humans.

Known as identical, monozygotic (MZ) twins are true reproductive clones. Like Dolly, they share a genome yet breathe a sovereign existence.

Through genetic studies in a large kindred with familial MZ twinning, a hitherto unknown hormone ELABELA (ELA) was characterized.

ELA is secreted by human embryonic stem cells and pre-implantations embryos, where it orchestrates pluripotency and possibly the birth of MZ twins.

After implantation, ELA drives cardiovascular development and may serve as a novel therapeutic for pre-eclampsia.

O-141 The biology of dizygotic twinning

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The biology of dizygotic twinning

Dorret I Boomsma, on behalf of the Twinning GWAS Consortium (TGC)[1*]
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Abstract

The spontaneous rate of twinning is about 1 in 80 live births, which means that about 1 in 40 individuals is a twin. In contrast to Monozygotic (MZ) twinning, Dizygotic (DZ) twinning has a familial component, with mothers of spontaneous DZ twins having a higher number of DZ twin pairs in their pedigrees than the general population. In Caucasians, two-thirds of twins are DZ and dichorionic diamniotic (DCDA), commonly referred to as fraternal twins since they develop from two separate oocytes fertilized at the same time. The underlying biological mechanism for DZ twinning is the release and fertilization of multiple oocytes, for which both animal models and human data suggest multifactorial inheritance. So far, efforts to characterize the genes that contribute to DZ twinning in humans and explain its high prevalence have not been successful. Candidate gene association studies and genome-wide linkage scans have failed to find common variants associated with DZ twinning.

We established the Twinning GWAS Consortium (TGC) to characterize the genetic basis of spontaneous DZ twinning and performed the first genome-wide association study (GWAS). We report for the first time compelling evidence that sequence variation at the FSHB and SMAD3 loci increases the odds of DZ twinning, based on 3 discovery cohorts from the Netherlands, Australia and Minnesota (USA). The findings were replicated in a large cohort from Iceland by Decode. We show that variants in these two genes are significantly associated with a broad range of fertility and reproductive traits in women including age at menarche, age at menopause, age at first and last child, and lifetime parity. We estimate the relative risk of twin birth given the novel alleles and explore the genetic relationship of DZ twinning and fertility traits using polygenic risk scores (PRSs), and show that higher DZ twinning PRS is associated with having children, greater lifetime parity, and earlier age at first child.

We expect substantial interest in the risk estimates for women, who are carriers of these variants, which we report based on population data from Iceland. We hypothesize that the results will also be of importance to investigations into ovarian response to follicle-stimulating hormone (FSH) stimulation for assisted reproductive technology (ART), making these findings relevant to research into female infertility.

Twinning is associated with common perinatal and maternal morbidities and by understanding the genetic basis of human DZ twinning we concomitantly identify loci conferring susceptibility (or conversely resistance) to these prevalent perinatal comorbidities.

Keywords: dizygotic twinning, fertility, genetics, GWAS polygenic risk scores

SELECTED ORAL COMMUNICATIONS

SESSION 39: PARAMEDICAL 3 - NURSING

Tuesday 05 July 2016 **Room 101** **11:45–12:45**

O-142 Reproductive options for transmen

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Study question: How do transmen (female-to-male transgender persons) and their female partners experience current and future reproductive options and how do they make decisions about it?
Summary answer: The couples experienced their reproductive options as very limited. Their positive attitude towards treatment with stem cell-derived gametes was linked to traditional family values.
What is known already: The Belgian law states that transgender people who want to change their legal sex have to undergo physical gender adjustment to the extent medically possible and justified. This means that they have to accept the fact that they can no longer reproduce. Yet, fertility preservation options do exist: oocyte freezing and ovarian tissue cryopreservation for female-to-male transgender people and sperm freezing for male-to-female patients. Also, Belgian transgender people can be considered for fertility treatment with donor gametes; they are not excluded on the basis of their background or sexual orientation.
Study design, size, duration: In this study, we included 6 female-to-male transgender people with their female partners. The in-depth semi-structured couple interviews were performed between September 2014 and January 2015. Four reproductive options were discussed: donor conception, adoption, oocyte freezing and stem cell-derived gametes. Data were analysed through step-by-step inductive thematic analysis. A continuous auditing process by the co-authors resulted in themes that were grounded in the data.
Participants/materials, setting, methods: The participants were recruited at the Department of Reproductive Medicine of a University Hospital. Three couples had at least one child as a result of donor insemination with anonymous donor sperm (the first-born was between 7 and 8 years old) and three were either in treatment or pregnant at the time of data collection. Approval of the clinic's Ethics Committee was obtained.
Main results and the role of chance: The couples viewed donor conception as the standard route to become parents. This was also influenced by the fact that the female partners had a strong wish to become pregnant. Adoption was seen as less obvious because of the transgender past and the fact that it was a time-consuming process.

Couples mostly talked about oocyte freezing as a hypothetical situation, since this had not been presented as an option at the time of their transition. Reasons for not wanting to freeze oocytes before the completion of the female-to-male transition were the transgender person's wish to close the chapter of his life in a female body as quickly as possible and the absence of a partner or the wish to become a parent. However, the possibility to have a genetic link with the child was perceived as a benefit of this technique. The importance of *both* parents having a genetic link with the child was reflected in their opinion about the hypothetical option of generating gametes from stem cells. Initial reactions of disbelief or astonishment changed quickly into a positive attitude towards this possible development.
Limitations, reasons for caution: Although we retrieved rich data, a sample size of six is rather small. However, inclusion criteria were adapted to be able to increase inclusion. Nevertheless, three couples refused participation, one did not respond after receiving information about the study and one couple could not be reached after several attempts.
Wider implications of the findings: This study illustrates the experience of female-to-male transgender people and their female partner about reproductive decision-making. Gametes generated from stem cells are a valuable option for these couples. However, transgender people should be counselled about all reproductive options at the time of the transition.
Trial registration number: NA.

O-143 Poor knowledge on age-related fertility decline and *in vitro* fertilization (IVF) among healthcare professionals outside of assisted reproductive technologies (ART)

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Study question: How knowledgeable are gynecologists, physicians of other specialties, and nurses about fertility, assisted reproduction techniques (ART), and social freezing?
Summary answer: Gynecologists are more knowledgeable about women's fertility and ART than other specialties physicians and nurses, but less open to social freezing.
What is known already: Healthcare professionals are the main source of fertility information and counseling for the general public. However, reproductive knowledge has been shown to be lower than desirable in medical students and female healthcare professionals (nurses, midwives and medical doctors). On the whole, healthcare professional are aware that age-related infertility exist, but are not able to pinpoint when fertility starts to decline and overestimate the chances of achieving a pregnancy (either natural or thorough ART). Conversely, while most healthcare professional agree that age related fertility needs to be discussed during women's annual revision, informing women about social freezing is still controversial.
Study design, size, duration: This cross-sectional study includes 201 healthcare professionals (72 gynecologists, 78 physicians in specialties other than gynecology, and 51 nurses) from 4 public hospitals in Spain. Participants filled in an anonymous survey about fertility, ART and social freezing.
Participants/materials, setting, methods: The survey included 10 questions on age-related infertility, ART (until what age can a woman get pregnant spontaneously/through IVF/through oocyte donation-OD-?), and attitude towards social freezing (should it be offered to every young woman/be financed by the public health system?). Responses among the 3 professional categories were compared by ANOVAs and Student's *t*-tests. Impact of responders' age, gender, having children, stable work and relationship on age-related infertility knowledge was further investigated by multivariate analysis.
Main results and the role of chance: Overall, the reported mean age limit for achieving a pregnancy spontaneously, through IVF, and through OD were 39.5 ± 4.5, 43.7 ± 5.2 and 49.0 ± 6.5 years, respectively. Gynecologists reported a significantly younger limit age for spontaneous pregnancy (37.5 ± 2.8) and for IVF pregnancy (41.0 ± 3.4) than other physicians and nurses ($p < 0.001$). Only 4.2% of gynecologists reported an age limit for a spontaneous pregnancy over 40 years, compared to 40.0% of other physicians and 27.1% of nurses, ($p < 0.001$). Similar results were found for the reported age limit for IVF and OD pregnancies. Gynecologists were more conservative regarding vitrification, with 41.8% considering that social freezing should be offered to every young woman, vs. 62.7% of other physicians and 48.9% of nurses ($p = 0.041$), and 19.4% considering that social freezing should be financed by the public health system (vs. 31.9% of other physicians and 42.2% of nurses, $p = 0.031$). Most gynecologists (47.1%) would not offer social freezing past 35, while 44.6% of other physicians and 57.8% of nurses would offer it until 40. Gynecologists were significantly younger and nurses significantly older and of female gender than the other groups, however the multivariate analysis did not show an effect of age or gender on participant's answers.
Limitations, reasons for caution: We should be aware than 41% of physicians other than gynecologists in our survey were family physicians, who could have a different average fertility knowledge compared to other specialties, since they are primary care physicians with a broad consultation mandate which includes family planning.
Wider implications **of the findings:** Age-related fertility knowledge is poor among healthcare professionals in Spain, and attitudes towards social freezing could be based on mistaken believes; both inaccurate knowledge and personal attitudes could influence the quality of information and counseling given to patients.
Trial registration number: NA.

O-144 Sex counselling for subfertile couples continuing to attempt natural conception

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Study question: How would subfertile couples continuing to attempt natural conception like to receive sex counseling?

Summary answer: Subfertile couples would prefer written over face-to-face sexual advice and additionally want advice on stress management, the menstrual cycle and life style behavior.
What is known already: Two underpowered randomized controlled trials (RCTs), suggest that face-to-face sex counseling improves clinical pregnancy rates of couples with unexplained subfertility. In other fields, improvement of sexual functioning of couples has been achieved with web-based sex-counseling. Web-based sex-counseling is less costly than face-to-face sex-counseling. Couples whom are advised "expectant management" (EM), i.e., who are sent home to continue attempting natural conception, are known to suffer from lack of information and support. Subfertile couples are known to value and attempt all strategies likely to improve their pregnancy chance.
Study design, size, duration: This study was conducted in the winter of 2015 and relied on explorative in-depth qualitative interviews with seven heterosexual subfertile couples.
Participants/materials, setting, methods: Couples with unexplained subfertility and a good prognosis on natural conception who underwent at least 6 months of EM were eligible. Couples were recruited from a Dutch and a Belgian fertility clinic. Ten couples were invited by telephone to be interviewed at the clinic or at home. The in-depth interviews were guided by open questions and a topic list and were recorded digitally. They were then transcribed verbatim and subjected to content analysis.
Main results and the role of chance: Seven couples were included and interviews were held with five couples and two women without their partners. All couples had received 6–9 months of EM. Five couples regretted delaying fertility treatment while two valued "giving nature another chance." During EM, all couples had timed intercourse and experienced diminished sexual pleasure. Although not having considered it, all couples could imagine benefitting from sexual advice, especially if it might improve their pregnancy chance. All but one couple preferred web-based over face-to-face sex counseling. Couples wanted to contact professionals *via* email with their questions. Couples did not agree whether they wanted professionals to initiate email contact. Written testimonials from other couples were preferred over interacting directly with them. Couples preferred sexual advice to be evidence-based, concise and endorsed by professionals rather than directly sexually stimulating. Drawings or cartoons were preferred over pictures. Sexual advice would be read, especially by women, and communication and sensate focus exercises would be attempted together. Uptake would have to be voluntary, with an advised rather than a compulsory sequence of exercises. Couples highly valued obtaining additional information on stress-management, the fertile window of the menstrual cycles and life style behavior affecting pregnancy chances.
Limitations, reasons for caution: Data-collection is ongoing as data saturation has not yet been achieved although it is expected by March 2016. Although the participation rate was high (70%), couples willing to participate in this type of interviews might be more positive about sexual advice than those declining participation.
Wider implications of the findings: Once data-saturation is achieved, our group will incorporate the insights from these patient interviews in a new web-based sex-counseling intervention. In a large scale RCT the effect of this intervention will be compared to EM regarding ongoing pregnancy rates, sexual and psychosocial functioning and will examine its feasibility and acceptability.
Trial registration number: Not applicable.

O-145 Comparison of patients – centeredness experience in infertility care in patients fully reimbursed by national insurance and in cross-border self-paying patients

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Study question: Is there a difference of patient-centered care (PCC) between national insurance reimbursed patients and cross-border, foreign infertility couples treated in public University clinic?
Summary answer: Slovenian and cross-border patients have different experience in all PCC domains, even though the overall satisfaction with infertility care is high in both groups.
What is known already: Patient-centered care is an important aspect of infertility treatment and high quality of care. Reports of patients' experiences vary across countries and this reflects the differences in individual patients' expectations, staff's competences and provider's organization of care. All EU members allows patients to seek cross-border infertility care but some limitations in reimbursement policy exist across countries. Staff's competences, success rate and out-of-pocket costs are some of drivers which guides patients' decision on choosing a hospital.
Study design, size, duration: This was a prospective, cross-sectional study and included randomly sampled couples who underwent fertility diagnostic procedure or ART in Slovenian fertility clinic during 12 weeks period. All Slovenian patients have six fully reimbursed cycles while cross-borders paid out-of-pocket.
Participants/materials, setting, methods: Difference in the level of patient-centredness was assessed between the groups of 106 Slovenian couples and 69 cross-border couples using a standardized questionnaire. Patient-centredness Questionnaire-Infertility (PCQ-Infertility), with a socio-demographic data included, comprises three assessment levels: total scale, seven domains and 46 single indicators (scale range 0–3). Women and their partners were recruited in clinical setting and fulfilled PCQ-Infertility questionnaire translated into two languages, Slovenian and SCB (Serbian–Croatian–Bosnian), and adapted for both groups of patients.
Main results and the role of chance: The majority of women in both groups (Slovenian 61.16%, cross-border 93.1%) were not pregnant at the moment of fulfilling the questionnaire after embryo transfer. At the domain level, the group of cross-border patients assessed all seven PCC domains higher than Slovenian patients. The biggest score domain gap (SDG) between two group was found in Continuity and transition (SDG 0.99) and Care Organization (SDG 0.84). The lowest SDG was in Accessibility domain (SDG 0.09). Unlike, Staff Competences domain was scored the highest in both groups. Based on the score indicators' gap (SIG), the results shows that Slovenian patients misjudged more indicators than cross-border patients, namely: staff member assigned as contact for any questions or problems (SIG 2.13), waiting time more than 3 weeks for an appointment with the physician (SIG 1.08) and the number of different physicians involved in treatment in the present hospital (SIG 1.54). Indicators that were assessed with the lowest score in both groups were information about psychological and social worker support and how often patients had impression that staff was speaking "about them" instead "to them."
Limitations, reasons for caution: Although we found sufficient response rates, higher number of participants would result in higher confidence of results. Because we only included infertile cross- border couples from ex-Yugoslav countries, we cannot draw conclusions that these results would be universally applicable to other cross-border couples treated in this particular clinic.
Wider implications of the findings: These results can present an incentive, in which we should put more focus on assessing the possible correlation between having a contact person in organisation (who speaks patients native language), number of doctors involved in treatment of each couple, and having a positive experience on continuity of care.
Trial registration number: NA, Future medicine, IPA Adriatic cross border cooperation project.

INVITED SESSION

SESSION 40: THE NEED FOR OXYGEN IN IVF

Tuesday 05 July 2016 **Hall 5 CB** **14:00–15:00**

O-146 Oxygen as a regulatory force during oogenesis and preimplantation embryogenesis: lessons for oocyte and embryo culture in clinical IVF

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Abstract text
The role of oxygen in oogenesis and preimplantation embryogenesis is usually considered in terms of cellular bioenergetics, i.e., ATP production *via* oxidative phosphorylation, and whether too low a level may compromise synthesis and one that is too high may be detrimental if associated with excess oxidative damage/stress. In clinical IVF however, oxygen concentration is one of the few

operator-controllable variables that can be easily applied to oocyte and embryo culture, with ranges between 5 and 20% (atmospheric) typical. While there have been numerous studies asking whether an optimal concentration exists with respect to normal fertilization, embryogenesis and outcome, most have concluded that levels in the lower range should be routinely used. Although *in vitro* culture conditions attempt to approximate *in vivo* conditions, what oxygen concentrations oocytes and embryos experience in the reproductive tract it is not well understood, nor is the basic question of whether possible changes in ambient oxygen within different regions normally occur that could have stage-specific regulatory influences on developmental competence.

This presentation focuses on what the likely oxygen milieu the oocyte and preimplantation stage embryo experiences within the mature preovulatory follicle, fallopian tube and uterus, respectively. While changes in oxygen concentration *in vitro* can be correlated with quantitative differences in levels of gene expression for certain classes of proteins, comparisons to *in vivo* developed embryos suggest these differences may be more "noise" than signal and likely developmentally benign. This raises the question of how oxygen levels are sensed and can elicit different molecular responses in the early embryo. What will be the focus of these findings is that while near anoxic conditions experienced by an oocyte during the terminal stages of preovulatory nuclear maturation can have negative downstream developmental consequences, the same conditions experienced by the peri-implantation blastocyst *in vivo* may be required to induce or upregulate the expression of certain growth factors likely needed during the invasive phase of implantation. That relatively small changes in ambient oxygen concentration from near anoxic for the oocyte to near anoxic for the embryo may be beneficial with respect to developmental competence may provide novel insights into the role of oxygen in oogenesis and early embryogenesis that may warrant consideration in the design of dynamic *in vitro* culture systems for clinical IVF that better approximate location-specific conditions *in vivo*.

O-147 Is oxygen really harmful to human embryo culture?

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Abstract text

Preimplantation embryos from different mammalian species have been shown to develop successfully *in vitro* when cultured under atmospheric (~20%) as well as under low (5–7%) oxygen concentrations. Furthermore, high implantation rates and development to birth are obtained with embryos cultured during the preimplantation period under varying oxygen concentrations. Also for human *in vitro* fertilization (IVF) treatments, both atmospheric as well as reduced (5%) O_2 concentrations are widely used and high success rates have been reported. Which oxygen concentration results in the best preimplantation development and in the highest implantation rates is subject for a wide range of studies performed during a period spanning almost five decades.

Theoretically, it can be assumed that culture under a low oxygen concentration is more physiological. Embryos develop *in vivo* under low O_2 levels, as in the oviduct and uterus of various mammalian species O_2 concentrations have been reported to vary between ~1.5–9%. In accordance with this fact, preimplantation embryo development *in vitro* in most animal models is shown to be significantly improved when the culture is performed in reduced oxygen concentrations in the majority of studies. Also in the human, significant improvement of development to the blastocyst stage and an increased number of cells per blastocyst was found in several studies when 5% O_2 was used. Regarding pregnancy and live birth rates however, the methodological quality of most published studies was relatively poor and the sample sizes were too small to detect the relatively small differences in results that were to be expected, resulting in a confusing maze of contradictory outcomes. It was only recently that results from systematic reviews and meta-analysis confirmed that culturing embryos under a low oxygen concentration improves the success rates of IVF and ICSI, when trials were considered in which embryos were transferred on days 5 and 6 at the blastocyst stage (Gomes Sobrinho et al., 2011; Bontekoe et al., 2012).

Besides the above mentioned outcome parameters it is important to realise that during recent years many studies have shown that exposure to atmospheric oxygen leads to a variety of adverse cellular effects ranging from significantly elevated levels of intracellular reactive oxygen species leading to increased apoptosis and DNA damage, altered gene expression and metabolic changes in preimplantation embryos. As is the case with success rates, also with these cellular effects contradictory results were found.

However, altogether, the results of all above mentioned studies provide growing evidence that the use of a reduced oxygen concentration is to be preferred for the culture of mammalian preimplantation embryos and for human *in vitro* fertilization (IVF) treatments.

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INVITED SESSION

SESSION 41: INSULIN SENSITIZERS IN REPRODUCTION

Tuesday 05 July 2016 **Hall 5 A** **14:00–15:00**

O-148 Role of metformin and AMPK in reproductive tissues

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Abstract text

Metformin is a drug widely used in the treatment of diabetes mellitus type 2 and can be also used to improve fertility in case of reproductive abnormalities associated with insulin resistance. Indeed, women with polycystic ovary syndrome (PCOS) are treated with clomiphene citrate associated or not with metformin to improve ovulation rate. Its action reduces hepatic glucose output, increases tissue insulin sensitivity and enhances peripheral glucose uptake. To clarify the mechanism of metformin's action in reproductive tissues, several studies have used direct and indirect exposures.

Observations mainly *in vivo* or in ovarian cell culture models have been described in several species (human, rodents, cow and goat) where reductions of estradiol, progesterone from the granulosa and even androgen by human thecal cells was observed *in vivo* and *in vitro* after metformin exposure. Metformin alters differently oocyte maturation in function of the species. Indeed, in bovine and porcine oocytes, metformin blocked meiotic progression to the germinal vesicle stage whereas opposite effects are observed in mice

During pregnancy, metformin has the ability to cross the placenta, and its use raises concerns about potential adverse effects on both the mother and fetus. Some studies have observed that *in utero* exposure to metformin early in pregnancy does not appear to cause any adverse effects or congenital malformations. Despite this, consequences on gonad development of the fetus have not been clearly studied. Hence, *in vitro* studies and *in vivo* experiments in mouse models have reported a decrease in androgens production by human and testis embryonic gonads. The free testosterone index (FTI), which is the ratio used to evaluate the androgen status in humans and is calculated by the total testosterone level divided by the sex hormone binding globulin (SHBG) level, was increased in metformin-exposed male offspring. In addition, an increase in levels in SHBG newborns exposed to metformin during the first trimester of pregnancy has also been reported.

In adult testis, metformin stimulation resulted in a 3-fold increase in lactate production that is an important energy source for male germ cells. Thus, metformin could impact directly male germ cells.

In conclusion, the data are still insufficient to completely confirm or disprove negative effects of metformin. In this review we will provide an overview of the various aspects of metformin in sexual reproduction, and the safety use of metformin.

O-149 Insulin sensitizers in the treatment of metabolic disturbances and infertility in PCOS

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Abstract text

Polycystic ovary syndrome (PCOS) affects 6–18% of women of reproductive age and is the most common cause of anovulatory infertility in women. The first-line treatment of the adverse features of PCOS is lifestyle intervention, such as weight loss and exercise. Reduction in weight of as little as 5% can restore regular menses and improve response to ovulation-inducing and fertility medications.

The intrinsic role played by insulin resistance and hyperinsulinemia in the pathogenesis of PCOS has led to the use of insulin-lowering drugs for the treatment of the syndrome. Numerous studies have indicated that metformin improves metabolic abnormalities, e.g., hyperinsulinemia and insulin resistance, decreases androgen levels and improves menstrual pattern in women with PCOS. Previously, several reports and meta-analyses have suggested that metformin may improve ovulation induction 1.5 to 4 times compared to placebo alone. Later, these results could not be confirmed in two large randomized studies, where metformin was significantly less effective than clomiphene, and the combination of the two drugs did not bring any advantages. In the most recent Cochrane meta-analysis the addition of metformin to an ovulation-induction agent significantly improved clinical pregnancy rates (PRs), but not live birth rates (LBRs). Two recent RCTs conducted in Nordic countries, however, showed that a pretreatment of 3 months with metformin improved the PRs and LBRs by 15% after IVF/ICSI in non-obese women, and after its combination with standard infertility treatment in obese women. Altogether, these findings support the use of pre-treatment with metformin for several months before infertility treatment. The last recommendation of the American Association of Clinical Endocrinologists, American College of Endocrinology and AE-PCOS Society is that metformin may be useful as an adjuvant agent in certain groups of women with PCOS, such as obese women.

The efficacy of metformin in addition to gonadotropin stimulation, laparoscopic drilling and/or IVF treatment remains to be defined. Recent studies suggest that metformin improves pregnancy outcome by decreasing early spontaneous miscarriage, but these findings could not be confirmed in recent randomized, prospective, placebo-controlled trials. However, metformin has been shown to decrease significantly the risk of ovary hyperstimulation syndrome during assisted reproductive technology.

Obese women with PCOS are at increased risk for metabolic syndrome (MetS) with impaired glucose tolerance (IGT: 31–35%) and type 2 diabetes mellitus (T2DM: 7.5–10%). Additionally, compared with BMI- and age-matched controls, young lean women with PCOS have lower serum levels of high-density lipoprotein (HDL) size, higher levels of low-density lipoprotein (LDL) and triglycerides. Metformin is efficient in improving weight loss and may prevent conversion of IGT to T2DM as well as reduce features of MetS from 35 to 21% of subjects. Metformin use has also been associated with improvement of dyslipidemia in women with PCOS. In adolescents with PCOS metformin may be used as first-line monotherapy or in combination with oral contraceptive pills to reduce PCOS symptoms.

Drugs in the insulin sensitizing thiazolidinedione class (i.e., pioglitazone) are effective for treating insulin resistance and hyperinsulinemia in PCOS. These drugs, however, are associated with potential serious side effects and are contraindicated during pregnancy and therefore cannot be recommended for use in PCOS.

Inositols are members of the vitamin B complex. They are important mediators of insulin action and therefore classified as "insulin sensitizers." Both isoforms of inositol, D-chiro-inositol and myo-inositol have been reported to improve insulin sensitivity, ovulatory function and hyperandrogenism in women with PCOS as well as pregnancy outcomes in infertile women with poor oocyte quality. However, large placebo controlled studies are needed to confirm these results before insitols can be recommended for the treatment of PCOS.

INVITED SESSION

SESSION 42: EMBRYO AND DOUBLE GAMETE DONATION (PATIENT SESSION)

Tuesday 05 July 2016 **Hall 3 AB** **14:00–15:00**

O-150 Should embryo donation be mandatory? Patients' perspective on Polish law on infertility treatment

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Abstract text

Should embryo donation be mandatory? Patients' perspective on Polish Law on infertility treatment.

IIn November 2015 the new law on infertility treatment was entered into force in Poland. According to its provisions, surplus embryos must not be destroyed or donated for scientific research. Each embryo with preserved development potential is now guaranteed to be transferred. For tens of thousands of couples who have surplus embryos but do not plan on having any more children this means that they are forced to transfer them despite their reproductive plans or to donate the embryos. The regulations apply to all existing embryos, including embryos created before the new law was enforced. The new regulations apply also to single women and lesbian couples who have no longer the right to undergo any ART or MAR treatment as it is strictly reserved for heterosexual couples. As a consequence single women and lesbian couples are no longer legitimate to transfer the embryos created from their eggs before November 2015. For this group of patients this means that the embryos they recognize as their offspring and are willing to transfer are taken away and dispose by the state. For all the patients who are not legitimate or are not willing to transfer or donate their cryopreserved embryos the above results in compulsory donation. After 20 years of cryo-preservation without their consent or even knowledge the embryos will be overtaken and disposed as so called "embryo adoption."

The new regulations do not seem to comply with the European Directive 2004/23/EU which provides for "voluntary and unpaid donations of tissues and cells." Polish legislative solutions on organ donation are unheard in any other European state. Nevertheless, the new law on infertility treatment has been announced in Polish media to be "a successful compromise between medical liberalism and bioethics."

As it was to be expected the said regulations reverberated in the patients circle. In the presentation I will outline how the Polish patients evaluate this solution and what social and ethical costs will be entailed for people undergoing ART technologies in the past and in the future.

O-151 Comparative analysis on the situation of embryo/double gamete donation in Romania and France

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Comparative analysis on the situation of embryo/double gamete donation in two European countries: (1) Romania: This country has no MAR legislation, but only a general one on health, which comprises strict provisions on human cells (as such, as regards double gamete donation, the cross-border reproductive care phenomenon unfolds, Czech Republic and Greece being preferred destinations), and as regards embryo donation, there is no legislation whatsoever.

(2) France: This country has MAR legislation, which forbids double gamete donation (for that type of procedure, the concerned persons use cross-border reproductive care) but allows embryo donation, and many clinics have information and encouragement campaigns for embryo donation.

O-152 Embryo and double gamete donation – talking and telling

C. Spencer[1]
[1]*Donor Conception Network, Head Office, London, UK*

In the UK, increasing numbers of couples and individuals are contemplating and proceeding with using double donation (sperm and egg) and/or using embryo donation (sperm and egg at embryo stage) in order to create or add to a family. It may be that women have reached an age where conception using their own eggs is simply not possible, or have other reasons why their own eggs cannot be used (e.g., genetic issues or premature ovarian failure).

Whatever the reasons for turning to double or embryo donation, couples and individuals then have to decide whether to tell any child/children about how they were conceived. The Donor Conception Network (DC Network) in the UK has worked for over 20 years to support families with children conceived through donor conception and believes openness with donor conceived children is good for the children and the whole family. Although some of the issues are very similar when telling any child they are donor conceived, there appear to be some subtle but important additional considerations, both for the parent and the child, that arise when talking and telling about double and embryo donation. These add an additional layer of complexity. How do you explain this

complicated information to a child under the age of five? How do you tell other people that this is how you conceived your child and what are their reactions likely to be?

This talk will include a personal story and professional experiences (Caroline is a psychotherapist and a facilitator of DC Network workshops as well as a mum) to illustrate how to talk to young children conceived through double and embryo donation about their origins. It will address how this talking may evolve as the child's cognitive ability develops and their interaction with the broader community expands. The talk will also cover the emotional factors that parents of double/embryo donation have to contend with such as their own feelings about having a child with no genetic connection to themselves as well as highlighting some of the key topics that come up in working with prospective and actual double and embryo donation parents.

Recent feedback from donor conceived children in the UK who are part of the DC Network tells us that although many of them feel well supported at home and within the family setting, at school and in the broader community this is not always the case. This talk will draw on both personal examples and professional knowledge to highlight why being appropriately open and honest in its broadest sense is so important in supporting children conceived this way as they grow up.

O-153 Embryo donation – new legislation – the case in Israel

O. Balaban-Kasztelanski[1]
[1]*CHEN-Patient Fertility Association, Holon, Israel*

Double donation/embryo donation – The role of NGOs – the Israeli case.

Non Profit Organizations have an important role in Health laws Promotion at the national health level and international level.

While having ministry of health on one side, Pharma industry on second side, professional doctors on third side – we need to look at the forth and most important actor – the patients that are *via* NGOs that have the most influence in Health laws Promotion.

In Israel, there is a strong and valid legislation with regulations regarding double donation.

Regarding embryo donation there is no legislation yet – we applied to the government to prepare it.

The law and regulations regarding double donation are very clear: If a woman need to have double donation she may get an egg donation from Israeli woman but the sperm must come from abroad. It is preferred to be a non Jewish donor.

The concern is not only religion, but genetically concern – to avoid donations that are close genetically. Women can have an egg donation in 7 out of 28 pubic clinics in Israel from an Israeli donor. The sperm must be from abroad as said. The state covered the cost of egg donation but not the price of the sperm. The patient has to pay 10,000 SHEKELS as part of the payment to the donor that is being done *via* the clinic. All the procedure must take place in the clinic.

Cross border is allowed and the state participates in the costs – about 30% is covered. In this case no regulation regarding the sperm or the egg origin.

Embryos donation – new legislation in Israel: In Israel there are more than 150,000 frozen embryos in the laboratories. This is unbearable situation since nothing can be done with it without the parents' permission.

It is high cost to keep them and the current law allowed only 3 options for the parents: (A) To keep the embryos. (B) To unfreeze the embryos. (C) To donate for research.

The option of donating the embryos to other person is not allowed by law.

We started a new legislation to allowed the forth option according the situation of England, Canada and France.

In Israel, the role of NGOs is so important that they keep the systems work. Without the volunteering organizations the system cannot function at all. We are changing laws for the benefit of the patients, we take care of implantation of the systems.

The problem in Israel is that by religion, it is not allowed to a married coupled to receive an embryo donation.

With consultation and cooperation of the ministry of health it was decided to start the procedure of legislation with the following restrictions: (1) The law will be as an adoption law and not donation (like other gamete donation).

(2) The recipient will be only a single woman – to avoid the problem of a married woman carried a pregnancy that does not contain her husband sperm.

(3) The anonymity situation in donation embryos/double gametes is still unclear. The adoption law allows the child to check about his genetically parents at age 18 years old. This issue has to be discussed.

We started the procedure that probably will take few years in the Israeli political situation.

With the cooperation of the ministry of health we gathered the government and the Kenesstt to work together for the preparation of the law. This is one of the strong abilities of NGO in Israel.

INVITED SESSION

SESSION 43: LEGISLATION AND ECONOMICS OF THE EU TISSUE & CELL LANDSCAPE

Tuesday 05 July 2016 **Hall 3 DE** **14:00–15:00**

O-154 Overview of EU legislation applicable for IVF/ART

O-155 Economics for IVF/ART

O-156 Exchange/Q&A on key concerns for the IVF/ART sector on the current EU legal framework

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INVITED SESSION

SESSION 44: PARAMEDICAL INVITED SESSION - NURSING

Tuesday 05 July 2016 **Room 101** **14:00–15:00**

O-157 Can nurses and midwives help patients cope with treatment?

H. Ockhuysen[1]
[1]*Nieuwegein, Netherlands*

Women often report that an IVF treatment is an emotional and physical burden that causes high levels of anxiety and stress. The moments most reported as stressful are oocyte retrieval, the 14-day waiting period between embryo transfer and pregnancy test, and being informed that the treatment was unsuccessful. Despite the emotional stress reported during fertility treatment, many women do not seek professional support. The stated reasons for this are often practical, such as costs of counselling, distance to the appointment or being unsure how to arrange an appointment. Although women do not often make use of professional psychosocial help, they expect that ongoing emotional support should be part of a fertility treatment delivered by nurses, midwives and doctors working in the fertility clinic.

Nurses have been working in fertility care since the first IVF baby was born and their specialised roles are continually developing and evolving. Nurses are a constant member of the fertility team and are usually more accessible than other members of the fertility clinic. This approachability gives nurse the opportunity to further develop the challenging task to support patients to cope with the several stages of an IVF treatment. Research shows that most fertility nurses regain knowledge for effective practice by experimental learning and professional development activities. One of the challenges in fertility nursing care is to build a bridge between evidence-based practice and daily practice.

This presentation will give a short overview of some evidence-based interventions and methods available for nurses to help patients cope with the several stages of an IVF treatment. Instruments like the SCREENIVF, to identify women or men at risk for emotional maladjustment before the start of a fertility treatment, or the Positive Reappraisal Coping Intervention, to promote the coping strategy positive reappraisal, are examples of evidence based intervention to help patients cope with treatment but also to improve and further develop fertility nursing care.

O-158 Three psychosocial interventions staff could incorporate in routine care today to support patients

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Fertility patients have psychosocial and medical needs. Medical needs are mostly well defined and addressed in myriad ways by clinical and paramedical staff. In contrast, psychosocial needs are often poorly defined and addressed primarily through referral to specialised mental health professionals (MHPs). This state of affairs has proved unsatisfactory for numerous reasons (e.g., patient reluctance to consult with MHPs, practical aspects of arranging consultations) with the most importance consequence being that patients often do not get the psychosocial care required to support them during treatment journey. Medical and paramedical staff members often do want to meet patient psychosocial needs but often lack clarity about what can be done, especially what can be done given the constraints of their workload and skill set.

ESHRE recently produced guidelines that can inform on how best to approach psychosocial care from the perspective of fertility clinic staff ("Routine psychosocial care in infertility and medically assisted reproduction: A guide for fertility staff"). The guidelines specify that to best help patients, staff must be empowered to detect and address psychosocial needs (e.g., behavioural, relational, social, emotional, cognitive needs) using effective assessment and interventions. One of the most important patient preferences expressed identified in the ESHRE guidelines was the need for effective information. In particular written, customised information about the treatment procedures and results, and access to psychosocial care. At the time of publication, the provision of information to fertility patients was the only psychosocial intervention found to be effective. In light of this finding and the constraints on staff time, one way that fertility clinic staff can support patients is through provision of information.

In this presentation Information provision in three topic areas that address patient needs will be discussed (quality of life, oocyte retrieval, expectation management). The presentation will illustrate what has been done and what can be done. Also discussed will be how fertility clinic staff can assess and, if needed, improve their own clinic documents using quality assessment tools designed to evaluate the provision of information.

SELECTED ORAL COMMUNICATIONS

SESSION 45: EMBRYO (EPI)GENETICS AND MIRNAS

Tuesday 05 July 2016 **Hall 1** **15:15–16:30**

O-159 The position of the inner cell mass during blastocyst biopsy does not affect clinical results

H. Blanca[1], J. Ten[1], M. Diaz[1], A. Rodriguez-Arnedo[1], J. Guerrero[1], J. Llacer[2], F. Sellers[2], R. Bernabeu[2]
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Study question: To evaluate the effect of the inner cell mass (ICM) position at the time of trophectoderm biopsy on clinical outcomes.
Summary answer: The position of the ICM at the time of the biopsy does not affect pregnancy and miscarriage rates.
What is known already: Trophoectoderm biopsy allows more accurate genetic diagnosis without apparent embryo damage. Assisted hatching (AH) prior to the biopsy is performed on day 3 because it promotes embryo hatching, making easier the removal of the cells. This process makes the ICM may be inside, outside or in the herniation in the moment of biopsy. Nothing is known about the relation between the position of the ICM and clinical results, such as clinical pregnancy or miscarriage rates.
Study design, size, duration: Prospective study. We include the known clinical results of 157 euploid embryos: 107 of them with the ICM inside (group I), 32 with the ICM outside (group II) and 18 of them with the ICM in the herniation (group III), transferred to 139 women that underwent comprehensive chromosome screening (CCS) treatments from January 2014 to October 2015.
Participants/materials, setting, methods: At least one euploid embryo was transferred to 139 patients. AH was performed on day 3 using laser pulses (Saturn Active, Research Instruments). On day 5 of development, a conventional trophectoderm biopsy was done. The position of the ICM in the moment of biopsy was recorded. Clinical outcomes were evaluated.
Main results and the role of chance: There was no statistically significant difference in the clinical pregnancy rate when the ICM was inside (group I), outside (group II) or in the herniation (group III) (41.3, 48.3 and 45.5%, respectively, $p = 0.794$). The position of the ICM did not affect biochemical miscarriage (10.9% in group I, 6.9% in group II and 16.7% in group III, $p = 0.638$) or clinical miscarriage (14.6% in group I, 10% in group II and 12.5% in group III, $p = 0.877$).
Limitations, reasons for caution: Study currently under development to increase the number of cases and test the study question.
Wider implications of the findings: These results confirm that the position of the ICM in the moment of trophectoderm biopsy does not affect clinical outcomes. In certain cases, where the ICM is outside the embryo, the maneuver during biopsy is more complicated, but this does not seem to affect the outcome of the embryo.
Trial registration number: N/A.

O-160 Direct unequal cleavages: embryo developmental competence, genetic constitution and clinical outcome

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Study question: What is the correlation between embryos with direct uneven cleavages (DUCS) and consequent developmental potential, chromosomal constitution and clinical outcome?
Summary answer: Blastocyst formation and embryo implantation were significantly reduced in embryos expressing DUCS. The euploid rate in DUCS embryos were lower compared to normally cleaving embryos.
What is known already: Published time-lapse studies indicated lower developmental and implantation potential of abnormally dividing (direct cleavage) embryos. The sample size of these studies were small, and only described abnormalities at the first embryo cleavage stage.
Study design, size, duration: A retrospective cohort study conducted from November 2011 to June 2014. A total of 21,261 embryos from 3,155 IVF cycles were analyzed.
Participants/materials, setting, methods: Only normally fertilized (2PN) embryos cultured in time-lapse incubators (EmbryoScope®, Vitrolife, Sweden) were included in the study. A total of 21,261 embryos from 3,155 cycles (2,471 ICSI and 684 standard IVF) were analyzed. DUCS embryos were classified into DUC-1 (first cleavage), DUC-2 (second cleavage), DUC-3 (third cleavage) or DUCSPlus (DUCS occurring more than once). Clinical outcome and PGS results were analyzed for DUCS.
Main results and the role of chance: The total incidence of DUCS were 26.1%: 9.78% in DUC-1, 9.06% in DUC-2, and 3.73% in DUC-3. The occurrence of DUCS were not correlated with female gamete age or source. The incidence of DUC-1 was significantly higher in embryos fertilized by epididymal and testicular sperm (13.6 and 11.4%, respectively) compared to ejaculated sperm (9.05%, all $p < 0.05$). DUC-1 incidence was also greater in IVF embryos (11.5%) vs. ICSI embryos (9.1%). The total incidence of DUCS were strongly correlated with the onset of blastomere multinucleation during the first three divisions. In DUCS embryos, the blastocyst formation rates gradually decreased from 40.2% (DUC-3), 18.8% (DUC-2), 8.2% (DUC-1) and 5.6% (DUCSPlus), $p < 0.001$. The D3 FH rate were 12.42% ($n = 3172$) in Non-DUCS, 6.3% ($n = 127$ ET) in DUC-3, and 2.7% ($n = 260$ ET) in DUC-2 embryos. Zero live births resulted from either DUC-1 ($n = 225$ ET) or DUCSPlus ($n = 100$ ET) embryos. Lower FH rates (33.3%) and similar LB rates (40%) were observed in transferred DUCS blastocyst compared to Non-DUCS. The EUP rate gradually increased from DUC-1, -2, -3 to Non-DUC (12.9, 18.2, 31.6, 43.6%, $p < 0.001$) in Day 3 biopsied embryos. Contrarily, the complex abnormalities (CxA) rate gradually decreased with DUCS onset stage (80.6, 47.7, 36.8, 27.1%; $p < 0.001$).
Limitations, reasons for caution: PGS result were based on varying chromosomal platforms; the mosaicism and ploidy could not be fully investigated due to the methodology limitation. Only good quality embryos were biopsied (day 3 or day 5) and might not reflect the true aneuploidy (ANU) prevalence in embryos.

Wider implications of the findings: DUCS embryos occurring in first two cleavage stages should be deselected from D3 transfers, but might still be considered for day 5 transfer if the embryos achieve good quality blastocyst.
Trial registration number: N/A.

O-161 Methylation level of H19/IGF2 differentially methylated region (DMR) in human blastocysts donated by fertile couples

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Study question: Considering the possibility of epigenetic errors due to assisted reproduction techniques (ART), this study investigated the DNA methylation status of H19/IGF2 DMR in human blastocyst.
Summary answer: This study showed that the mean methylation status of H19/IGF2 DMR in high quality human blastocyst donated by healthy couples is around 37.85 ± 4.87%.
What is known already: Imprinted genes are a unique subset of few genes, which have been differentially methylated in a parental-origin dependent manner during gametogenesis. Although imprinted genes are specifically protected from the genome wide waves of epigenetic erasure and rebuild occur during pre-implantation embryo development, increasing evidence suggest particular vulnerability of some differentially methylated region (DMR) to interruptions often incur by assisted reproduction techniques (ART). In this study, we used high quality blastocyst donated by healthy couples without any infertility problem or poor-quality embryos to evaluate the epigenetic risks linked to ART.
Study design, size, duration: A total of 20 ICSI-derived blastocysts were donated from 7 different couples with at least two children of the same sex referring for family balancing. All embryos were collected after obtaining the written informed consents signed by couples. Blastocysts were scored according to Gardner et al. (2012) grading system. The peripheral human lymphocytes were used to validation of our technique. The embryos were collected from 2013 to 2015.
Participants/materials, setting, methods: Only blastocysts with A/B quality were selected. Methylation levels of H19/IGF2 DMR in expanded or expanding blastocysts were analyzed by bisulfite conversion and sequencing at 18 CpG sites (CpGs) located in this region. Methylation status of lymphocyte was used to obtain the pattern of imprinted gene methylation in somatic cells. More than 15 clones were sequenced per replicate.
Main results and the role of chance: Results showed that the overall percentage of methylated CpGs and the proportion of hyper-methylated clones of H19/IGF2 DMR in analyzed blastocysts were 37.85 ± 4.87 and 43.75 ± 5.1%, respectively. For validation of our technique the corresponding methylation levels of peripheral human lymphocytes were defined (49.52 ± 1.86 and 50%, respectively). We observed that the methylation status of H19/IGF2 DMR in human blastocysts is lower than the expected methylation level of somatic cells.
Limitations, reasons for caution: It has been stated that small starting amounts of template material, may create amplification bias.
Wider implications of the findings: Since in this study the high grade blastocysts were derived from fertile couples, one possibility are more likely to justify the cause of the observed methylation level: methylation status of DMR are not rigid during pre-implantation development embryo and can be affected at least in part by the genome-wide demethylation
Trial registration number: 1.

O-162 Comprehensive characterization of the human blastocyst's miRNome from the Inner Cell Mass, the Trophectoderm and their related IVF spent culture media

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[3]GENETYX, Molecular Genetic Laboratory, Marostica, Italy

Study question: From which section of the human blastocyst are miRNAs secreted in spent culture media (SBM) during IVF cycles?
Summary answer: MicroRNAs in SBMs derive mainly from the trophectoderm (TE) and in part also from the inner cell mass (ICM), as consistently confirmed with multiple qPCR platforms.
What is known already: MicroRNAs are non-coding RNA molecules acting as regulators of gene expression. They can be secreted in the extracellular environment and act upon recipient cells. They seem to be involved in the blastocyst-endometrium dialogue aimed at implantation, and mediate the autocrine communication between the cells composing the blastocyst as well. We recently profiled secreted-miRNAs in SBMs during IVF. They are secreted from the time of blastulation onwards, and 2 miRNAs (miR-20a-5p, miR-30c-5p) are significantly more abundant in SBMs from implanted versus unimplanted blastocysts. MicroRNAs are putative biomarkers of implantation and it is crucial to define whether they originate from ICM and/or the TE.
Study design, size, duration: Experimental study conducted at an IVF clinic (London, UK) between 4/2014 and 12/2015 and licensed by the NHS Research Ethics Committee. 10 excellent quality blastocysts (donated from consenting couples) and blanks were used. MiRNome analyses were conducted according to the TaqMan Low-Density Array cards protocol (TLDA protocol; LifeTechnologies, USA) human panel A + B, and confirmed through the microRNA PCR Human panel I + II (EXIQON protocol; EXIQON, Denmark) and single assays at a molecular biology laboratory (Marostica, Italy). 85% of TLDA and EXIQON panels (n = 754 assays) coincide.
Participants/materials, setting, methods: After warming, 10 blastocysts were singularly cultured in 25 µl-microdrops of Quinn's Advantage blastocyst medium (ORIGIO, USA) in a humidified atmosphere (5% O_2, 6% CO_2) for 12 h. After SBM collection, the ICM was biopsied using a previously validated method. 5 Trios (ICM + TE + SBM) were processed with the TLDA protocol (magnetic beads-based extraction + targeted retrotranscription + preamplification + qPCR; validity criteria: Ct ≤35 in ≥3/5 ICMs/TEs/SBMs). Data were confirmed on the other 5 Trios through the EXIQON protocol (column chromatography-based extraction + universal retrotranscription + LNA primers – SYBR Green-based qPCR; validity criteria: Ct ≤39 in ≥3/5 ICMs/TEs/SBMs).
Main results and the role of chance: The raw data Pearson's correlation according to the TLDA protocol ranged 0.82–0.87 within biological groups (only-ICMs/only-TEs/only-SBMs) and 0.74–0.83 between them. 133 miRNAs (range 127–146) were found valid in the TEs (overall mean Ct: 29.0 ± 4.1, range 13.3–34.9), 64 (72–83) in the ICM (29.3 ± 3.6, 13.0–34.9) and 71 (65–91) in the SBMs (30.0 ± 3.9, 13.9–34.9) according to the TLDA protocol. 49 miRNAs were exclusively expressed by the TEs, 13 and 20 were shared only with the ICMs and the SBMs, respectively. No miRNA was found solely in ICMs or SBMs. 51 miRNAs were shared by all the three biological groups. 55.0% (n = 28/51; 95% CI = 41.35–68.65%) of these last miRNAs were confirmed and validated through the EXIQON protocol.

A pathway analysis was conducted through the DIANA-miRPath software. 91 pathways were significantly predicted to be regulated by these 28 blastocyst-specific miRNAs: 38.5% involved in biosynthesis, apoptosis and differentiation, 28.6% involved in cell signaling and communication, 19.8% in cell growth and cancer and 13.2% in inflammation and angiogenesis.

We performed a differential expression of the normalized data in TEs versus ICMs. 10 and 1 miRNAs were found significantly ($p < 0.05$) upregulated in the ICMs (fold-change range 8–60.7) and in the TEs (fold-change 14.8), respectively. These miRNAs are all involved in promotion/inhibition of cell growth and/or stress-response by directly/indirectly influencing cell cycle regulation and apoptosis.
Limitations, reasons for caution: Due to a different layout, not all miRNAs within the TLDA panels could be confirmed by the independent EXIQON protocol. However, most of the miRNAs common to both the platforms showed a good consistency, as confirmed also through single assays. Only excellent quality blastocysts and relative SBMs were analyzed.
Wider implications of the findings: In this study we outlined the cellular origin of the miRNAs in SBMs and their possible roles within the blastocyst and in the blastocyst-endometrium dialogue. Furthermore, these data represent an initial step to define how miRNAs influence the mechanisms of differentiation/loss of pluripotency at this stage of preimplantation development.
Trial registration number: None.

O-163 MicroRNAs in spent blastocyst culture media: assays have to be improved

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Study question: Is it possible to detect and measure microRNAS (miRNA) in blastocyst spent culture media with enough sensitivity, specificity and reproducibility to use them as biomarkers of implantation potential?
Summary answer: MicroRNAs purification and detection methods have to be improved to fit with small amount of miRNAs and small sample volumes before being used in ART.
What is known already: MiRNAs are now well known to be involved in regulation of gene expression. Human embryos have been shown to secrete miRNAs that can be detected in IVF culture media. It has also been reported in preliminary studies that some of these miRNAs were differentially expressed according to the fertilization method, embryo chromosomal status, and pregnancy outcome. Those miRNAs are likely to be secreted by the trophectoderm.
Study design, size, duration: Spent blastocyst culture media (G2 Plus, Vitrolife) from single transferred blastocyst were prospectively collected on the day of embryo transfer (day 5 or 6 post fertilization) and immediately frozen at –80°C. Sixteen of them were randomly selected to be analyzed. Each sample was analyzed in duplicate.
Participants/materials, setting, methods: Mir-25 and mir-191, two miRNAs previously described as being present in embryo culture media, were assayed after phenol/guanidine-based lysis of samples and silica-membrane–based purification of total RNA (2 eluates for each sample) followed by Reverse Transcription and real time Sybr-Green qPCR (QIAGEN). A Nanodrop (Thermo Fischer) was used to quantify total RNA extraction. DNA samples migration (LabChipGX, PerkinElmer) was then analyzed to validate the specificity of DNA amplification and detection.
Main results and the role of chance: Total RNA extraction led to a mean total amount of 10.91 ± 7.23 ng/µL of RNA in culture medium. The mean CT values for miRNA detection were 33.80 ± 1.71 whereas CT values for blank samples were 58. Two duplicates had more than one gap CT value. Mir-25 and mir-191 had both a good quality detection aspect on the melt-curves for most of the samples. However, only few samples for mir-191 detection and none of the samples for mir-25 detection showed a specific strip (around 65 bp) after migration on a nanochip (Caliper). These results suggest that the assay methods have to be improved to be efficient enough to detect small amount on miRNAs in small volumes and used in clinical research.
Limitations, reasons for caution: Only one Sybr-Green assay has been tested: Taqman technologies and other purification methods may give better results.
Wider implications of the findings: The use of miRNAs as biomarkers of embryo viability and implantation potential still needs to be improved before being used as a way to improve assisted reproductive technologies outcomes.
Trial registration number: None.

SELECTED ORAL COMMUNICATIONS

SESSION 46: THE UTERUS IN INFERTILITY

Tuesday 05 July 2016 **Hall 5 CB** **15:15–16:30**

O-164 Regeneration of endometrial function such as establishment of pregnancy by endometrial cell sheet transplantation

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[3]*Tokyo Women's Medical University, Department of Obstetrics and Gynecology, Tokyo, Japan*

Study question: Can a regenerative-medicine technique using cell sheets become a new treatment method for endometrial disorder causing female infertility?
Summary answer: Regeneration of the endometrium following the transplant of an endometrial cell sheet created through cell sheet engineering led to a confirmed establishment of pregnancy.
What is known already: Endometrial disorder, i.e., Asherman's syndrome can cause infertility due to severe endometrial adhesions caused by severe endometrial defects including stem cells defects, therefore general therapy such as surgical synechiotomy is ineffective.

A new approach called "cell sheet engineering," which can harvest confluent-culture-cells as a contiguous cell sheet with intact cell–cell junctions and extracellular matrix without enzymatic treatment, has been developed for tissue regeneration.
Study design, size, duration: The control group only underwent resection of the endometrium, whilst the transplantation group received a transplant of a green-fluorescent-protein (GFP) positive cell sheet into the resected endometrium site. The groups were compared using macroscopic findings and histological analysis including immunohistochemistry. Following that, cross breeding was performed to confirm establishment of pregnancy in the regenerated uterus.
Participants/materials, setting, methods: GFP positive endometrial tissues were treated with trypsin/EDTA. Two kinds of cell sheets (epithelium and stroma) were produced from the isolated cells separated by pre-plate method, harvested from temperature-responsive-cell-culture dishes by reducing the temperature. Layered cell sheets were assembled from the two kinds of cell sheets, and transplanted into the endometrial resected site. The regenerated uterus was evaluated for the existence of GFP positive, and establishment of pregnancy was evaluated by ultrasound after crossbreeding.
Main results and the role of chance: At 4 weeks after surgery, in the transplanted cell sheet group, a wide GFP positive site was found. On the other hand, in the control group, a wide area of occlusion was found. In a histological analysis, a large GFP positive area was found, and luminal structure was confirmed uniformly. Endometrial glands were found with CK18 and GFP in a similar to normal uterine structure in the stromal layer.

After cross breeding, a GFP positive gestational sac (GS) was found in the transplantation group. On the other hand, in the control group, no GS was found. And in the cross section of the GS and in a histological analysis, a wide and circumferential area of GFP positive tissue was found.
Limitations, reasons for caution: The results are difficult to apply directly to humans, because the structure and function of rat uteri are different from those of humans.
Wider implications of the findings: Transplantation of endometrial-cell-sheets have a high possibility not only to prevent intrauterine re-adhesion after synechiotomy, but also to regenerate the endometrium. The regenerated endometrial tissues might have a normal function such as menstruation and implantation of a fertilized egg.
Trial registration number: Not applicable.

O-165 Should patients be immobilised after intrauterine insemination? A randomised controlled comparison between 15 min of immobilisation and direct mobilisation

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Study question: Does 15 min of immobilisation after intrauterine insemination (IUI) effect pregnancy rates?
Summary answer: Immobilisation of 15 min after IUI does not effect pregnancy rates.
What is known already: IUI is an established treatment for couples with mild male or idiopathic subfertility. It has been shown that several factors are related to pregnancy outcomes, however up to now no consensus exists on whether direct mobilisation is beneficial for pregnancy outcomes. Two recent studies report a beneficial effect of immobilisation, though these studies can be criticised due to a lack of power or low overall pregnancy rates and a variation of the use of ovarian stimulation. Besides, the potential responsible mechanism for the benefit of immobilisation remains unclear.
Study design, size, duration: Single center randomised controlled trial, based in an academic setting. 500 patients were approached between August 2010 and May 2014. To account for an increase of 2–3% per cycle of the ongoing pregnancy rate by immobilisation [as shown by Custers et al. (2009)], the study was calculated to include 229 patients per study-arm (alpha of 5% and beta of 80%).

Participants/materials, setting, methods: 479 patients diagnosed with idiopathic or mild male subfertility and an indication for IUI (1934 cycles) were randomised. Randomisation was stratified for the diagnosis idiopathic or mild male subfertility. 236 participants were assigned to 15 min of immobilisation (950 cycles), and 243 participants to immediate mobilisation (984 cycles). The primary outcome was the cumulative ongoing pregnancy rate per couple.
Main results and the role of chance: Patients characteristics were comparable between the two groups, except for the duration of subfertility [2.4 years (IQR 1.8–3.6) in the immobilisation group, 2.1 years (IQR 1.6–3.0) in the mobilisation group (*p*-value 0.017)]. The cumulative ongoing pregnancy rate per couple was not significantly different between the two groups: in total, 76 ongoing pregnancies (32.2%) were accomplished after 15 min of immobilisation versus 98 ongoing pregnancies (40.3%) after immediate mobilisation [OR 0.70 (95% CI 0.483–1.022), *p*-value 0.065)]. The odds ratio, after correcting for duration of subfertility, did not reach statistical significance either: OR 0.72 (95% CI 0.491–1.044, *p*-value 0.082). The Kaplan–Meier survival curves for time to pregnancy were not found to be different for the immobilisation and mobilisation group (median survival time 15.1 versus 11.7 months, *p*-value log-rank test 0.11).
Limitations, reasons for caution: Due to discontinuation of the treatment (social reasons, treatment alteration) not all participants reached six IUI cycles or an ongoing pregnancy, however this is as expected in IUI treatment and it mirrors clinical practice. These participants were equally distributed over the two groups.
Wider implications of the findings: This study shows that immobilisation after IUI has no positive effect on pregnancy rates. It even tends to the opposite. In our opinion, there is no reason why patients should stay immobilised for 15 min after IUI. This result is in disagreement with the literature, a meta-analysis is needed.
Trial registration number: Dutch Trial Register, NTR 2418.

O-166 Uterine fluid-derived extracellular vesicles as a new non-invasive tool to assess endometrial features

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Study question: Do extracellular vesicles (EVs) isolated from uterine fluid contain endometrial status biomarkers?
Summary answer: Molecular characterization of uterine fluid-derived EVs reveals a number of biomolecules (proteins, mRNA, DNA) that could be useful to enhance "liquid biopsy" performance.
What is known already: The potential value of uterine fluid and of its contents as biomarkers of different aspect of human endometrial pathophysiology has been well recognized but so far its use has been limited by the restricted amount of fluid available. For the first time uterine fluid-derived EVs were identified by Hunt Ng et al. (2013). EVs are a heterogeneous group of nanoparticles (~30–1000 nm) that are secreted by a variety of cell types, representing a possible vehicle of clinical and biological information. From a diagnostic standpoint, levels of EVs or their specific content have been recognized as markers for different diseases/physiological mechanisms.
Study design, size, duration: Uterine fluids were aspirated through an IVF transfer catheter from a total of $n = 15$ reproductive age patients undergoing diagnostic hysteroscopy. Experiments have been performed collecting samples of 2–3 µL of uterine fluid or obtained by lavage with 2.5 mL of sterile saline.
Participants/materials, setting, methods: Uterine fluid samples were subjected to differential standard ultracentrifugation to isolate EVs. The nature of the EVs was demonstrated using transmission electron microscopy and by western blot analysis using specific EVs biomarkers. Total RNA from EVs was analyzed by 2100 Bioanalyzer. Amplification of mRNA of genes involved in endometrial pathophysiology was done. A fragment of the mitochondrial MT-7S D-loop region and of the RNAse P gene was specifically amplified.
Main results and the role of chance: Our explorative analysis confirm previous information and indicate that uterine fluid is extremely rich in EVs population, composed by exosomes (30–150 nm) and microvesicles (150–1000 nm). These results tend to support the possibility to isolate a consistent amount of EVs-derived biological material from few microliters of uterine fluid. Importantly, EVs in uterine fluid contain large amounts of multiple and heterogeneous RNA species, that could be used to study levels of expression of genes known in endometrial pathophysiology. Slight quantity of ribosomal RNA (28S and 18S) was detected in the samples analyzed. The PCR amplifications indicate a strong presence of Beta-Actin (ACTB), Cyclooxygenase-2 (COX2) and Wingless-Type MMTV Integration Site Family, Member 4 (WNT4) transcripts and of Aromatase (CYP19) transcripts traces in both exosomal and microvesicular population. These genes are involved in different important endometrial physiological and pathological process such as proliferation, differentiation, inflammation and endometriosis. Interestingly for its rarity, gDNA and mtDNA were detectable in all the uterine fluid-derived EVs tested. Take together these preliminary results help us to bio-characterize the EVs from uterine fluid in order to better understand the extracellular signals carried by uterine fluid.
Limitations, reasons for caution: This is an explorative analysis of a pilot study.
Wider implications of the findings: This study supports the possibility to isolate a consistent amount of uterine fluid-derived EVS from few microliters. The potential use of non-invasive endometrial signals as a "liquid biopsy" represents the novelty of this study. This would potentially allow reducing surgical interventions currently performed to establish endometrial dysfunctions and fertility parameters.
Trial registration number: No clinical trial.

O-167 Controlled ovarian Hyperstimulation (COH) and intrauterine insemination (IUI) vs. *In Vitro* Fertilisation (IVF) for the first line treatment of unexplained subfertility – a randomised controlled trial

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Study question: What is the best first line management option to the treatment of unexplained subfertility, Controlled Ovarian Hyperstimulation (COH, with gonadotrophin) and Intrauterine Insemination (IUI) or *In Vitro* Fertilisation (IVF)?
Summary answer: Results suggest that three cycles of COH (with gonadotrophin) + IUI is as effective as one cycle of IVF with comparable on-going, multiple pregnancy rates.
What is known already: Debate continues whether IVF should be the sole treatment for couples with unexplained subfertility. The 2013 NICE fertility guideline recommends not routinely offering IUI to these couples but proceeding directly to IVF after 2 years of subfertility. The literature indicates that although the per-cycle success rate of IUI is lower than that of IVF, cumulative IUI success rates are comparable to IVF. IUI remains less invasive, less stressful and less time consuming than IVF, with better perinatal outcome for singletons. Only few RCTs have conducted head to head comparison of IUI + COH and IVF in unexplained sub fertility.
Study design, size, duration: This is a single centre parallel group randomised controlled trial (RCT) with balanced randomisation (1:1), conducted in a tertiary referral centre in London, UK. Between June 2013 and June 2015 we recruited 207 couples with unexplained subfertility. They were randomly allocated to three cycles of COH (with gonadotrophin) + IUI ($n = 101$) or one cycle of IVF ($n = 106$).
Participants/materials, setting, methods: Couples with subfertility of minimum 1-year duration, where the female partner was ovulatory, aged 23–38 years, both tubes patent, without any uterine abnormality and the male partner with normal semen parameters (WHO criteria), were recruited. Though the primary outcome was live birth/couple, we report here the on-going pregnancy rate (as the delivery of all participants is not yet complete). Outcomes were analysed according to intention to treat.
Main results and the role of chance: The mean age of the female partner was 31.9 years (SD 3.3), mean BMI 23.6 (SD 3.01), mean AMH of 22.04 (SD 15.8) and mean AFC 16.5 (SD 8.2). The mean duration of subfertility was 3.02 years

(SD 1.8). Out of 101 couples allocated to COH (with gonadotrophin) + IUI, 90 couples started their first cycle and 69 couples completed all three cycles and the on-going pregnancy rate per couple was 22 out of 101 (21.8%), and the cumulative conception rate 31.9%. Out of 106 couples allocated to IVF group, 81 completed one cycle and the on-going pregnancy rate per couple was 36 out of 106 (33.9%). The relative risk of pregnancy was 0.64 (95% CI 0.40–1.01) in IUI + COH compared to IVF. The multiple pregnancy rates per on-going pregnancy were 9.1% after COH + IUI and 8.3% after IVF. All multiple pregnancies in the COH + IUI group were twins. There were one set of triplets and two sets of twins in the IVF group. Out of 207 couples, 18 conceived spontaneously before or in between treatments and 17 couples had on-going pregnancies (8.2% per couple). There were 3 OHSS cases in the IVF group (2.8%).

Limitations, reasons for caution: These are preliminary result based on on-going pregnancy rates without, as yet, the live birth data for all pregnancies.

Wider implications of the findings: The results of this prospective RCT rules out any clinically important benefit of IVF over COH + IUI for unexplained subfertility, with similar on-going and multiple pregnancy rates between the two treatments. This questions the wisdom of directing couples with unexplained subfertility directly to IVF instead of first employing COH + IUI.

Trial registration number: ISRCTN43430382.

O-168 Proteomic assessment of the endometrial fluid the day of embryo transfer as a prognostic factor of implantation in *In Vitro* Fertilization

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[3]*IVI Bilbao, Human Reproduction Unit, Bilbao, Spain*

Study question: To evaluate the influence of the proteomic composition of endometrial fluid at embryo transfer in implantation in *In Vitro* Fertilization (IVF) programs.

Summary answer: Endometrial fluid provides information of clinical relevance concerning embryo implantation. Obtaining the endometrial fluid by aspiration at embryo transfer does not impair IVF results.

What is known already: The implantation is a dynamic process that occurs between the blastocyst and endometrium, and an inadequate endometrium can be considered as a determining factor in IVF failure. Endometrial tissue has been studied by several methods and a large number of proteins and other molecules are expressed in the endometrium. Our research group has developed a non-invasive approach to analyse endometrial markers of receptivity in endometrial fluid aspirated (EAF) during the window of implantation

Study design, size, duration: It is a non therapeutical intervention study, without changes in the clinical or pharmacological protocol, except for performing an aspiration of endometrial fluid at the moment of embryo transfer.

For the safety analysis, the control group was constituted by the patients assisted at our unit where EAF was not performed

Participants/materials, setting, methods: The study population consisted of 600 patients subjected to IVF at our center during a 18-month period. They were subjected to standard IVF/ICSI according to our protocol except the performance of endometrial fluid aspiration.

The endometrial fluid sample was aspirated from uterine cavity (50–100 µl) by introducing an "embryo-transfer" cannula and stored at −80°C.

For extraction and identification of the endometrium proteome "electrophoresis 2D" and "mass spectrometry" were performed.

Main results and the role of chance: Differential proteomic pattern was observed in patients achieving pregnancy (A group) and those whom pregnancy had not been achieved (B group).

We identified 31 proteins regulated differentially; fourteen of them are development, cytoskeleton and chaperon-related proteins.

The majority of these proteins were downregulated (n = 10), most of them with 0.4–0.7 fold change in relative abundance between both groups. However, some other proteins (n = 4) were overexpressed with more than 1.5-fold change.

Concerning safety, pregnancy rates were compared between patients subjected to endometrial fluid aspiration (EAF) and patients assisted at our Unit during the same period of time whom EAF was not performed. The rates were similar in both cases, the women subjected to EAF aspiration (33%) as the women whom EAF was not performed (30%).

Limitations, reasons for caution: With the available technology the endometrial fluid and its proteomic analysis (PA-EFA) require a highly complex laboratory and their results are not helpful to modify the embryo transfer policy. Developing a technology that could produce our results in a short period of hours would be useful.

Wider implications of the findings: Endometrial fluid aspiration followed by proteomic assessment could be an important tool in the decision process to ascertain the number of embryos to be transferred as well as the day of the cycle to perform the embryo transfer (ET), or even to delay ET to a later cycle.

Trial registration number: CIC-2010092.

SELECTED ORAL COMMUNICATIONS

SESSION 47: TROPHOBLAST AND ENDOMETRIAL CROSS TALK

Tuesday 05 July 2016 **Hall 5 A** **15:15–16:30**

O-169 The sirtuin 1 activator resveratrol modulates the retinoic acid signaling pathway and inhibits the expression of decidual markers in primary human endometrial stromal cells

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Study question: To investigate the impact of resveratrol on decidualization and the retinoic acid (RA) signaling pathway of human endometrial stromal cells (HESCs) *in vitro*.

Summary answer: Resveratrol induced PPARbeta/delta and PPARgamma and suppressed CRABP2 and RARalpha expression, however, suppressed decidual markers (PRL and IGFBP1) in differentiating HESCs

What is known already: Our previous study has shown that RA signaling pathway plays a significant role in balancing differentiation and apoptosis upon decidualization of the peri-implantation endometrium. The polyphenolic compound resveratrol is a natural activator of SIRT1 and protects against oxidative stress and cellular senescence *via* various signaling cascades, including the RA signaling pathway. Clinically, resveratrol may potentially be useful to protect against ovarian aging, although its effect on endometrial decidualization is not known.

Study design, size, duration: This study was approved by the Local Ethics Committee of Juntendo University, Faculty of Medicine (No. 14-103). Timed endometrial biopsies (LH + 7–11) were processed for primary HESC cultures.

Participants/materials, setting, methods: Primary HESCs were decidualized with 8-bromo-cAMP, progesterone in the presence or absence of resveratrol (100 µM). Various molecular techniques, including immunostaining, RTQ-PCR, and Western blot analysis, were employed to define the role of resveratrol treatment.

Main results and the role of chance: Treatment of HESCs with resveratrol increased the expression of SIRT1. Decidualization of HESCs in the presence of resveratrol selectively inhibited the expression of the RA-binding protein, CRABP2, and RA receptor, RARalpha (associated with apoptosis induction) but induced the expression of the alternative RA receptors, PPARbeta/delta and PPARgamma (associated with cell differentiation). However, resveratrol also inhibited the induction of classical decidual markers, such as PRL and IGFBP1. Transient treatment of HESCs with resveratrol prior to the addition of 8-bromo-cAMP and progesterone did not affect the expression of decidual markers.

Limitations, reasons for caution: *In vitro* findings do not necessarily reflect the complex *in vivo* situation.

Wider implications of the findings: Resveratrol treatment may potentially be useful to attenuate ovarian aging and improve oocyte quality but our findings suggest that it may also adversely impact on implantation by interfering with the decidual transformation of the endometrium. Furthermore, optimal decidual transformation of primary cultures may involve apoptosis or senescence of specific subpopulations.

Trial registration number: None.

O-170 Mono-2-ethylhexyl Phthalate inhibits human extravillous trophoblast invasion *via* PPARγ pathway

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Study question: Is the invasive potential of the human extravillous trophoblast cell influenced by mono-2-ethylhexyl phthalate (MEHP) – the primary metabolism of Di-(2-ethylhexyl) phthalate (DEHP)?
Summary answer: MEHP exposure inhibited the invasion of extravillous trophoblast *via* peroxisome proliferator-activated receptor γ (PPARγ) even at a concentration found in humans.
What is known already: Di-(2-ethylhexyl) phthalate (DEHP) is one of the most common environmental contaminants. Exposure to DEHP has been associated with early pregnancy loss in humans; however, the involved mechanism remains unclear and the invasive function of extravillous trophoblast exposed to mono-2-ethylhexyl phthalate (MEHP), the primary metabolism of DEHP, have rarely been studied. Extravillous trophoblast invasion is an important physiological step during embryo implantation in early development. However, there is no data concerning the effects of MEHP, the active metabolite of DEHP, on the extravillous trophoblast invasion.
Study design, size, duration: This study was designed to investigate the invasion of the human extravillous trophoblast cell line HTR-8/SVneo treated by MEHP and the underlying mechanism.
Participants/materials, setting, methods: Human extravillous trophoblast cell line HTR-8/SVneo cells were exposed to different concentrations of MEHP (1, 10, 100, 200 and 400 μM). Cell invasion was evaluated using Matrigel-coated transwell chambers and the expression of matrix metalloproteinases (MMPs) and their inhibitors (tissue inhibitor matrix metalloproteinases, TIMPs) involved in cell invasion were analyzed. Both pharmacological inhibitors and shRNA knockdown were used to determine the potential molecular targets responsible for the MEHP-inhibited trophoblast invasion.
Main results and the role of chance: After exposed to MEHP (1, 10, 100, 200 and 400 μM), the invasion index of HTR-8/SVneo was reduced to 89.1 ± 11.1, 74.4 ± 3.6, 64.7 ± 6.9, 62.3 ± 1.4 and 49.1 ± 7.8% of the DMSO control in the 1, 10, 100, 200 and 400 μM MEHP exposure groups, respectively. The concentration of MEHP that significantly inhibited trophoblast invasion was as low as 10 μM ($p < 0.05$), which was at a similar level to the MEHP concentration found in humans reported in previous studies. Down-regulation of MMP-9 expression by MEHP further supported its inhibition of HTR-8/SVneo invasion at the molecular level. Inactivation of PPARγ by either pharmacological inhibitors or shRNA knockdown blocked the MEHP-induced effects on HTR-8/SVneo invasion, accompanying with the recovery of inhibited MMP-9 expression.
Limitations, reasons for caution: Although HTR-8/SVneo cells are functionally similar to primary human trophoblast and widely used to study the invasive behavior of human trophoblast *in vitro*, there may be different between these cells and their *in vivo* counterpart such as gene expression.
Wider implications of the findings: The present study provides the first evidence that MEHP exposure inhibited the invasive function of human trophoblast cells even at a concentration found in humans. These findings may contribute to the DEHP-associated early pregnancy loss, and provided the novel evidence of the potential influence of phthalate on female reproduction.
Trial registration number: There is no trial registration number.

O-171 G-CSF increases Treg cell recruitment to the decidua during the first trimester in women with a history of recurrent miscarriage: possible involvement of β-hCG chemoattraction

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Study question: The goal of the study was to determine if G-CSF treatment affects regulatory T (Treg) cell recruitment to the decidua during the first trimester.
Summary answer: Our results show that G-CSF enhances normal Treg cell emigration from the peripheral blood, reflecting increased migration of these cells to the decidua.
What is known already: The key role of antigen-specific Treg cells in regulating tolerance to the semi-allogenic fetus in mammals is now well established. Published studies disagree however regarding whether Treg cells increase or decrease in peripheral blood of pregnant human subjects, and the effects of immunomodulatory agents on Treg cells in pregnancy remain largely unknown. A better understanding of Treg cells dynamic during human pregnancy may provide an opportunity for monitoring of immunological function and effects of immunomodulatory agents in pregnant patients.
Study design, size, duration: This retrospective study included women with a history of recurrent miscarriage (RM) treated with G-CSF (Neupogen; "Group B," $N = 74$) or other treatment (Intralipid and/or prednisone; "Group A," $N = 82$).
Participants/materials, setting, methods: Treatment was initiated at ovulation and discontinued at week 12 of pregnancy. Blood was drawn and levels of total white blood cells (WBCs), Treg cells, β-hCG and G-CSF were assessed at various time points (T_0 = pre-pregnancy; T_1 = 4–5 weeks; T_2 = 6–9 weeks; T_3 = 10–12 weeks; T_4 = 13–17 weeks). Treg cells were identified as $CD3^+CD4^+CD25^{hi}CD127^{lo}FoxP3^+$ by flow cytometry.
Main results and the role of chance: Relative to T_0 levels, Treg cells decreased in peripheral blood by 22% at T_1, 39% at T_2, and 42% at T_3 before rebounding back to 50% by T_4 in Group A. G-CSF significantly enhanced the decrease in Treg cell levels at T_1 and T_2, with Treg cells decreasing by 61% ($p < 0.001$) and 56% ($p = 0.09$) at these time points. The G-CSF enhanced the decrease in peripheral blood Treg cells, that was greater at T_1 in patients with ongoing pregnancies as compared with those that subsequently miscarried. Levels of WBCs increased in Group A at T_1–T_4, which was amplified by G-CSF, and levels of WBCs and Treg cells were strongly inversely correlated at all time points in both groups ($R = -0.98$ in Group A and $R = -0.8$ in Group B). Additionally, b-hCG levels were increased in G-CSF-treated patients and were inversely correlated with peripheral blood Treg cell levels. These data confirm previous studies showing a decrease in peripheral blood Treg cell levels during the first trimester due to recruitment to the decidua. The data further show an enhancement of this migration by treatment with G-CSF which is correlated with increased levels of β-hCG, a known chemoattractant for Treg cells which express the LH/CG receptor.
Limitations, reasons for caution: x.
Wider implications of the findings: Increased recruitment of Treg cells to the decidua by G-CSF suggests a mechanism by which this treatment promotes fetal tolerance and pregnancy maintenance in women with a history of RM. We propose a model whereby G-CSF increases trophoblast β-hCG production leading to increased Treg cell chemoattraction to the decidua.
Trial registration number: x.

O-172 Endometrial receptivity and miscarriages: crucial roles of endometrial microRNAs

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Study question: Are there miRNAs in endometrial tissue during the implantation windows predicting early miscarriage after fresh or frozen embryo replacement?
Summary answer: We identified 126 miRNAs differentially expressed in receptive endometrium between pregnant patients ending in a live birth compared with those who have had a miscarriage.
What is known already: The rates of miscarriages within the framework of the replacement of fresh or frozen-thawed embryos in patients under hormone replacement therapy remain a major problem in IVF/ICSI. However, to date, there are extremely few studies analyzing molecular signature in endometrial tissue during the implantation windows in pregnant patients associated to miscarriages.
Study design, size, duration: Endometrial biopsies ($n = 10$) were collected during the implantation windows under hormone replacement therapy

(6–9 days after progesterone administration). Then RNAs were extracted for mRNA and miRNA purification to perform RT-qPCR gene expression and the miRNA expression profile, respectively. The RT-qPCR gene expression consists of measuring the expression level of 13 transcripts associated to the endometrial receptivity by RT-qPCR (Window Implantation Test; Patent EP10305561.2).
Participants/materials, setting, methods: Endometrial biopsies were obtained from 10 patients with repeated implantation failure (≥3). The endometrial receptivity status was asserted. Fresh or frozen-thawed embryo replacement has been performed according to the qRT PCR transcriptomic approach result allowing successful pregnancy and live birth. miRNA expression profiles between the two groups of pregnant patients ending with a miscarriage (n = 5) between 8 and 12 weeks period of amenorrhoea and live birth (n = 5), were evaluated with the *Affymetrix® miRNA 4.1 Array Strips.*
Main results and the role of chance: Using 3 distinct statistical analyses (Student's t test, Wilcoxon signed-rank test and ANOVA), we identified 126 miRNAs differentially expressed pregnant patients achieving live birth versus a miscarriage. These 126 miRNAs were all over-expressed in endometrium analysed during the implantation windows of subsequent pregnant patients succeeding to a miscarriage. Using the Ingenuity software, we first aimed to identify the potential he potential target genes of these microRNAs. We identified 28 microRNAs that are putative regulators of 11,062 genes attached to numerous biological functions including the leukocyte extravasation signalling, the regulation of the epithelial–mesenchymal transition pathway, the complement system, the chemokine signalling, the epithelial adherent junction signalling and the growth hormone signalling that play a crucial in implantation and maintain of pregnancy.
Limitations, reasons for caution: The number of samples used for microRNA analyses are low. However, the relevance of these biomarkers is being validated in independent large cohort of patients.
Wider implications of the findings: The identification of endometrial miRNAs associated to a miscarriage opens new perspectives in patient care management. In addition, it would be possible to select strategies by which miRNA technologies (antagomiRs, agomiRs) might be utilized in novel, non-hormonal therapeutic approaches to avoid miscarriages and consequently, to increase the pregnancy rate.
Trial registration number: Not applicable.

O-173 NLRP7 mutations in spontaneous abortions with multilocus methylation defects at imprinted genes from woman with recurrent pregnancy loss

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Study question: Is there association between mutations in *NLRP7* and multilocus methylation defects (MLMD) in imprinted genes in spontaneous abortions (SA) from women with recurrent pregnancy loss?
Summary answer: We found the association between mutations in *NLRP7* and MLMD in imprinted genes in SA from women with recurrent pregnancy loss.
What is known already: Previously we have reported that the MLMD at imprinted genes may cause karyotypically normal miscarriage in natural cycle, particularly among women experiencing recurrent miscarriage (Lepshin et al., 2014). The *NLRP7*, *NLRP2* and *ZPF57* genes have maternal effect that is essential for maintaining of genomic imprinting (Messerschmidt et al., 2012). In addition, mutations in *NLRP7* were associated with MLMD in imprinted genes at biparental complete hydatidiform mole (BiCHM).
Study design, size, duration: We have been sequenced all 11 exons of *NLRP7* gene in 36 SA with MLMD in two or more imprinted genes: 29 SA from women with recurrent miscarriage and 7 SA from women with sporadic miscarriage. Epimutations were found in *PEG1*, *DLK1*, *PEG10*, *PLAGL1*, *KvDMR*, *PEG3* and *GRB10* genes.
Participants/materials, setting, methods: All spontaneous abortions had the normal karyotype. We performed the direct sequencing of the *NLRP7* exons in SA with MLMD of imprinted genes.
Main results and the role of chance: We found mutations in the *NLRP7* gene in 27% SA from women with recurrent miscarriage (8/29). Moreover, all embryos with mutations in *NLRP7* had three or more methylation defects at imprinted genes. Mutations in *NLRP7* were identified as compound heterozygous or heterozygous state. Most of mutations represent missense mutation in exons 3, 4, 6 and 7, which lead to amino acid substitution. In addition, we identified one embryo with single nucleotide deletion, and another embryo with single nucleotide insertion in exon 7, that leads to a frame-shift.
Limitations, reasons for caution: Today, some other genes (*NLRP2*, ZPF57) are associated with MLMD at imprinted genes. However, in this study, we analyzed gene mutations only in *NLRP7*.
Wider implications of the findings: Mutations in *NLRP7* were detected only among SA with MLMD in imprinted genes from women with recurrent miscarriage that suggest the presence of the mutations in heterozygous state in these couples. Thus, mutations in the *NLRP7* associated not only with the BiCHM, but also with recurrent pregnancy loss.
Trial registration number: Not applicable.

SELECTED ORAL COMMUNICATIONS

SESSION 48: SAFETY AND QUALITY IN ART 1

Tuesday 05 July 2016 **Hall 3 AB** **15:15–16:30**

O-174 Double embryo transfer (DET): just for women taller than 1.65 m?

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[5]*Maternidade Dr. Alfredo da Costa, Materno-fetal Medicine, Lisbon, Portugal*

Study question: Should maternal height in nulliparous women be taken in to account when opting for DET in ART treatments?
Summary answer: Possibly. We observed that a maternal height ≥165 cm positively influenced (statistically significant) the obstetric results of twin pregnancies in nulliparous women.
What is known already: In a study by Dickey et al., the authors found an inverse correlation between maternal height and the risk of preterm births. Maternal height 176 cm was associated with a 14% reduction in the preterm birth rate in twins (statistically significant). An increased risk of preterm birth for women shorter than 150 cm was statistically significant in singletons, but not in twins. A different study by Blickstein et al. analyzed triplets and found that taller women (>165 cm) were more likely to deliver heavier triplets and were at lower risk of delivering triplets with very low birth weight.
Study design, size, duration: Retrospective cohort study evaluating pregnancies followed at Maternity Dr. Alfredo Da Costa – CHLC, between 1995 and 2015. Of the 2200 pregnancies followed in that period, 754 nulliparous women with dichorionic pregnancies were selected. Two groups were considered: (A) maternal height ≥165 cm (N = 271), and (B) maternal height <165 cm (N = 483). The following obstetric results were compared: gestational age at birth, delivery <28 weeks, delivery <32 weeks, average weight of newborns, newborns with weight at birth <1500 and <2500 g.
Participants/materials, setting, methods: Obstetrical outcome of nulliparous women with dichorionic-diamniotic pregnancies (N = 754). Women with height ≥165 cm (N = 271) versus women with height <165 cm (N = 483).
Main results and the role of chance: No statistically significant differences in maternal age (30.7 ± 4.8 versus 30.7 ± 5.2), pregnancy etiology (spontaneous versus ART) and mean BMI (23.0 ± 4.1 versus 22.8 ± 4.2 kg/m^2). Statistically significant differences in mean gestation age at birth (35.8 ± 2.8 weeks (wks) versus 34.8 ± 3.6 weeks, p < 0.001), delivery <28 weeks (4/271 (1.5%) versus 24/483 (5.0%), p = 0.01; delivery <32 weeks 12/271 (4.4%) versus 54/483 (11%), p < 0.001), average birth weight of newborns (2369 ± 444 versus 2179 ± 553 g, p < 0.001), newborns <1500 g (3.9 versus 10.9%, p < 0.001), newborns <2500 g (59.2 versus 68.7%, p < 0.001). With respect to complications during pregnancy, the only statistically significant differences found were in the rate of preterm labor (39.9 versus 47.8%, p = 0.03). No statistically significant differences between the two groups were found with respect to hypertension disorders and gestational diabetes.
Limitations, reasons for caution: Only nulliparous women were considered, so the findings may not apply to multiparous women.

Wider implications of the findings: Previous studies had already suggested that, in triplets, maternal height >165 cm was positively associated with gestational age at birth. According to our study, the same is true for twins. Increased pregnancy risk in nulliparous women shorter than 165 cm should be taken into consideration in double embryo transfers.
Trial registration number: We do not have a trial registration number.

O-175 Critical appraisal of the content of high quality clinical practice guidelines for fertility preservation

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Study question: What are the recommendations of the best clinical practice guidelines (CPGs) on fertility preservation (FP) in young women?
Summary answer: Seventeen relevant topics were identified in seven high quality CPGs. Two topics were discussed in all seven CPGs, while four were discussed by only one.
What is known already: It is vital that women who have to undergo gonadotoxic treatment and want to secure their fertility receive the best possible care related to FP. Current FP care, however, is far from optimal. One way to facilitate moving towards optimal care is by developing high quality CPGs which can guide clinicians in caring for their patients. The quality of a CPG may influence the quality of the recommendations made and indirectly the quality of care.
Study design, size, duration: An extensive literature search to identify all (inter)national CPGs on the subject of FP care in young women was conducted in February 2015 and updated in July 2015. A total of 32 CPGs were included and appraised for methodological quality (MQ) by four appraisers each. The content of the CPGs that scored high for their "rigour of development," was extracted and studied. The recommendations were categorised according to different phases in FP care.
Participants/materials, setting, methods: The list of CPGs found was reviewed for missing CPGs by an (inter)national panel of experts in the field of reproductive medicine or FP. 32 experts appraised the MQ of the included CPGs by using the Appraisal of Guidelines for Research and Evaluation (AGREE)-II Instrument. The content of the CPGs scoring >60% of points for their "rigour of development" was extracted independently by two members of the research team.
Main results and the role of chance: Of the total of 1,808 documents found, 30 CPGs were included into our study. After consulting (inter)national experts, two additional CPGs were included. Seven of these 32 CPGs scored high on MQ. These were the CPGs developed by the ASCO, the COSA, the NVOG, Italian Hematology Society, the RCOG, the SIGN and the NICE-RCOG. Five out of these seven guidelines were developed by European organisations.

The 17 identified topics of the recommendations provided by these seven CPGs were categorised in one of the following phases in FP care: diagnosis, discussion with oncological healthcare provider, referral, discussion with reproductive medicine specialist, FP treatment procedure, gonadotoxic treatment phase, fertility follow-up, (counselling) about wish to conceive, pregnancy and long term consequences. CPGs published prior to 2013 lack to discuss topics as reproduction after gonadotoxic treatment in contrast to CPGs published from 2013 on. Furthermore, the CPGs developed before 2013 discuss less topics (4–8 out of 17) than the CPGs developed after 2013 (7–12 out of 17). While the most complete CPG discussed 12 topics (ASCO), another CPG (COSA) provided the most recommendations for the most number of topics (5 or more recommendations on four topics) discussed.
Limitations, reasons for caution: Documents with recommendations published by established organisations were included and graded as if they were CPGs, which might be a limitation. Although we conducted an extensive search, CPGs may have been missed. Finally, CPGs were assessed on MQ, but the medical quality of the content was not taken into account.
Wider implications of the findings: Healthcare providers can use the extracted recommendations of the best CPGs in daily practice right away. The recommendations found can also be used for the development of quality indicators which will be helpful to monitor the quality of current FP care or to evaluate improvement initiatives.
Trial registration number: –.

O-176 Oocyte retrieval simulator: a safe and efficient tool for training

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Study question: The aim of this study was to establish the usefulness of an oocyte retrieval simulator (PICKUPSIM™, Accurate srl) for training gynaecologists in basic oocyte pick-up techniques.
Summary answer: The PICKUPSIM proves to be an adequate and safe training tool for oocyte pick-up, for both novice and non-novice gynaecologists.
What is known already: Oocyte retrieval is a common surgical procedure in assisted reproduction treatment. Standard practice is currently to perform a recommended number of procedures under supervision, until the trainee reaches proficiency, but this approach is not tailored on the individual nor safe for the patient. On the contrary, simulator-based training may provide a safe and controlled environment for learning basic skills.
Study design, size, duration: Prospective, single-centre study at Ferrara University Hospital including a total of 13 participants (5 "non novices," accounting for Group A and 8 "novices" accounting for Group B) who performed two simulation sessions 1 week apart from each other.
Participants/materials, setting, methods: The PICKUPSIM™ is a box simulator, equipped with a transvaginal probe, a needle and a foot pedal pump for aspiration of the follicles. The haptic feedback simulates the resistance to penetration of the soft tissues traversed by the Ovum Aspiration Needle, in particular the ovarian surface and the ovarian follicle. Scenarios are based on real clinical images and displayed on a wide-view monitor. The participants also completed a questionnaire on the training system.
Main results and the role of chance: The non-novices (Group A) aspired a significantly higher number of follicles in the first try, but proved to be generally slower than the novices (Group B). As to the second try, Group A showed better results in terms of time, while keeping constant the average number of aspired follicles. The novices significantly increased the average number of follicles aspired, reaching results comparable to those of Group A and slightly reduced the medium time needed to complete the procedure. Overall, the two groups improved their performances both in terms of time and efficiency. Most of the participants (81.8%) considered the box simulator useful for the training and for improving hand-eye coordination and wanted to use it for training in the future. They also agreed that the feedback from the trainer during the training course was very useful. Accordingly, 72.7% participants wanted to attend an oocyte retrieval training course using the box simulator in the next future.
Limitations, reasons for caution: The small sample size limits the power of our study.
Wider implications of the findings: The PICKUPSIM™ allows acquiring basic skills in oocyte pick-up without risk to patients. Simulator-based training should be considered for developing knowledge as an adjunct to more traditional training on real patients.
Trial registration number: No.

O-177 Coasting (withholding gonadotrophins) for preventing ovarian hyperstimulation syndrome

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Study question: To assess the effect of withholding gonadotrophins (coasting) on the prevention of ovarian hyperstimulation syndrome (OHSS) in assisted reproduction cycles.
Summary answer: This updated systematic review shows there is evidence of a benefit of using coasting compared with no coasting to prevent moderate to severe OHSS.
What is known already: Previous systematic review suggest there was no evidence of a difference in the incidence of moderate and severe OHSS, live birth or in the clinical pregnancy rate between costing versus no coasting or coasting versus other interventions such as early unilateral follicular aspiration or gonadotrophin-releasing hormone agonist (GRHa). Significantly fewer oocytes were retrieved in coasting groups compared with GnRHa or no coasting.
Study design, size, duration: For the update of this Cochrane systematic review we conducted a search according to the Cochrane Gynaecology and Fertility search strategy for randomised controlled trials (RCTs) in which coasting

was used to prevent OHSS from inception to July 2015. Twenty four studies were identified and six met inclusion criteria. Two review authors independently selected trials and extracted data.
Participants/materials, setting, methods: Only randomised controlled trials (RCTs) in which coasting was used to prevent OHSS were included. The intervention comparisons were coasting versus no coasting or early unilateral follicular aspiration (EUFA) or GnRHa or follicle stimulating hormone administration with HCG trigger (FSH co-trigger). The inclusion criteria for coasting was when estradiol levels were >2500 pg/mL or >9000 pmol/L. Statistical analysis was performed in accordance with the Cochrane Gynaecology and subfertility Group guidelines.
Main results and the role of chance: There was a significant difference in the incidence of OHSS [odds ratio (OR) 0.11, 95% CI 0.05–0.24] in the coasting groups compared to no coasting and in the coasting group compared to the FSH co-trigger group (OR 43.74, CI 2.54–754.58). There was no evidence of a difference in the other intervention groups. There was no significant difference in clinical pregnancy rate in all groups. Significantly fewer oocytes were retrieved in coasting groups compared with no coasting or the other interventions.
Limitations, reasons for caution: There was heterogeneity between the trials and several of the trials were only published as conference abstracts. There may be other relevant data which have not been reported (LBR, implantation rate). The results of the systematic review must therefore be interpreted with caution.
Wider implications of the findings: Coasting remains beneficial at preventing moderate to severe OHSS without affecting clinical outcomes. However there is emerging evidence that other interventions such as FSH administration with HCG trigger may prevent OHSS but more studies are needed to investigate this further.
Trial registration number: Not a trial.

O-178 Interventions for the prevention of ovarian hyperstimulation syndrome (OHSS) in assisted reproductive technology (ART) cycles: an overview of Cochrane reviews

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Study question: What is the current evidence on the prevention of OHSS in ART cycles published in the Cochrane library?
Summary answer: Twenty-seven, mainly high-quality, Cochrane reviews reporting on OHSS were included, of which nine showed evidence of significant reduction in OHSS between interventions.
What is known already: OHSS is a serious and potentially life-threatening complication of ART cycles, with an estimated incidence of 3–8%. This incidence is even higher in groups of high risk patients, such as women with polycystic ovary syndrome (PCOS), women with excessive follicle growth, a large number of retrieved follicles or high Estradiol levels during stimulation. In the Cochrane Database of Systematic Reviews, there are four reviews describing interventions for the prevention of OHSS. Other Cochrane reviews on ART cycles also report on OHSS as a secondary outcome and may help to identify potentially useful interventions to prevent OHSS.
Study design, size, duration: A systematic search of the Cochrane Database of Systematic Reviews was performed in November 2015 to identify all available systematic reviews of randomised controlled trials (RCTs) that report on the incidence and prevention of OHSS in ART cycles as a primary or secondary outcome. We identified published systematic reviews and review protocols.
Participants/materials, setting, methods: All ART reviews that report on OHSS as a primary or secondary outcome were eligible for inclusion. For each review, characteristics were summarised: number of included studies and patients, outcome reporting, timing of intervention, main limitations, effect size and quality of evidence. The GRADE (Grading of Recommendations Assessment, Development and Evaluation) and AMSTAR (A Measurement Tool to Assess Systematic Reviews) tools were used to assess the quality of included evidence and reviews respectively.
Main results and the role of chance: In total, 27 Cochrane reviews reporting on OHSS (variously defined) as a clinical outcome could be identified, including 87,340 patients. As these reviews followed the Cochrane standards of reporting, the methodological quality, as assessed by AMSTAR, was high. Nine of the interventions showed evidence of a reduction in the rate of OHSS (e.g., metformin adjuvant therapy in PCOS women, use of Gonadotrophin-releasing hormone antagonists and use of progesterone for luteal support). For 13 intervention reviews however, there was no difference in OHSS rates between intervention and control groups. In general, GRADE ratings showed that the available evidence was of very low to moderate quality. This was mainly due to failure to include any or only few studies per comparison and a low proportion of primary studies reporting data on OHSS. Of the reviews that did report on OHSS, three only reported on "severe OHSS," and 21 only reported on "total OHSS," which includes the clinically less important cases of "mild OHSS." This heterogeneous method of reporting fails to truly recognise the incidence of the clinically important moderate and severe cases, resulting in an evidence gap on this important adverse outcome of ART cycles.
Limitations, reasons for caution: Only 12/27 reviews were updated in the past 3 years, whereas nine were being updated at the time of our search. Reviews were hampered by poor reporting of subgroups "moderate" and "severe" OHSS in primary studies, making comparisons between interventions difficult.
Wider implications of the findings: There is a need to improve the reporting of OHSS as an outcome in RCTs on ART cycles. We advise trial investigators to report on the subgroups "moderate" and "severe" OHSS separately in future RCTs, as this provides more clinically useful information than merely reporting on "total" and "severe" OHSS.
Trial registration number: Not applicable.

SELECTED ORAL COMMUNICATIONS

SESSION 49: STEM CELLS

Tuesday 05 July 2016 **Hall 3 DE** **15:15–16:30**

O-179 Influence of female age on expression of mesenchymal stem cell-related markers and differentiation potential of aspirated follicular cells derived from women in the IVF programme

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Study question: Do mesenchymal stem cell-specific characteristics of aspirated follicular cells in follicular fluid depend on age of women included in the *in vitro* fertilization (IVF) programme?
Summary answer: The increasing female age doesn't affect the expression of mesenchymal stem cell-related markers of aspirated follicular cells, while it negatively correlates with their differentiation potential.
What is known already: In the IVF procedure ovarian follicular fluid aspirates are daily discarded after oocyte pick-up. In these follicular aspirates granulosa cells (GCs) represent the majority of cells, along with blood and epithelial cells. They play an important role in growth and development of ovarian follicle. Moreover, they contain a subpopulation of GCs expressing some stem cell characteristics. When isolated from follicular aspirates, they can be maintained *in vitro* for prolonged periods of time, can express mesenchymal stem cell (MSC)-related markers and are able to differentiate *in vitro* into different cell types.
Study design, size, duration: Altogether 12 samples of follicular aspirates were collected from 12 women included in the IVF programme at our department. Women belong to four different age groups consisted of three women each: (1) ≤30 years (Group A), (2) 31–35 years (Group B), (3) 36–39 years (Group C) and (4) 40–43 years (Group D). From follicular fluid of each woman cell culture was established to study the effect of female age on stemness of follicular cells.
Participants/materials, setting, methods: The aspirated follicular cells were isolated from follicular fluid with hypoosmotic technique and cultured in DMEM/F12 culture medium, supplemented with 15% of fetal bovine serum and 0.003 IU/mL of FSH. Cultured cells were analyzed with Human Mesenchymal Stem Cell RT^2 Profiler PCR Array and immunocytochemistry to evaluate the expression of MSC-related markers. After exposure of cultured follicular

cells to different induction media differentiation of cells into adipogenic and osteogenic lineage was confirmed by tissue-specific staining.
Main results and the **role of chance:** Most of follicular cell cultures were able to grow *in vitro* for 1.5 months (3–4 passages, splitting ratio 1:3) and few of them were even able to proliferate for 3 months (10 passages). The immunocytochemistry revealed that aspirated follicular cells expressed MSCs-related markers H-CAM, M-CAM, STRO-1, and CD90, regardless of female age. There was no difference in the proportion of positive cells between different age groups, possibly due to two reasons: first, in all groups 80–90% of cells were positive for H-CAM and 5–10% of cells for STRO-1, and, secondly, high variability for CD90 and M-CAM-positivity was observed within groups (0–90% for CD90 and 10–80% for M-CAM) because of unknown reason. *In vitro* cultured follicular cells from women of all age groups were able to differentiate into adipogenic cells. Although, the proportion of differentiated cells depends on female age: in Groups A and B more then 50% of cells differentiated, while in Groups C and D this proportion was smaller (5–25%). Interestingly, osteogenic differentiation was observed only in young women of Group A. Gene expression analysis confirmed the expression of above mentioned and other tested MSCs-related markers (CD105, CD73, CD166, CD340, VCAM1) although these genes were down-regulated in comparison to bone-marrow derived MSCs.
Limitations, reasons for caution: A higher number of samples should be included in each group due to high variability of samples, a common issue in stem cell research.
Wider implications of the findings: The properties of follicular cells in follicular fluid aspirates could be correlated with quality of corresponding oocytes and outcome of IVF treatment. Because these cells are discarded in medical practice, further studies are needed to evaluate, if they have a potential to be used for cell therapies in regenerative medicine.
Trial registration number: /.

O-180 Massive parallel sequencing uncovers chromosomal mosaicism in human embryonic stem cells in both primed and naive culture conditions

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Study question: Can massive parallel sequencing reliably detect copy number variations (CNVs) at high specificity, sensitivity and resolution in human embryonic stem cell (hESC) cultures?
Summary answer: Shallow whole-genome sequencing of hESC lines down to the single cell level efficiently identified CNVs as small as 4 Mb and revealed genetically heterogeneous cultures.
What is known already: HESCs harbour a propensity for genomic instability in culture. Numerous aberrations have been reported, from whole-chromosome aneuploidies to CNVs, ranging from a few kb to several Mb in size. Nonetheless, studies have been restricted to bulk analysis of DNA obtained from copious cells, which limits detection of low-abundance variants, masked by the genetic heterogeneity of cultures. With the advent of single cell whole-genome sequencing, preliminary insights into the mosaic nature of hESCs have been attained. However, all current data are exclusive to primed hESCs. With various naive conversion protocols recently established, high-resolution whole-genome characterisation of naive hESCs is paramount.
Study design, size, duration: Shallow whole-genome sequencing was performed on bulk cell (>200,000 cells), 3–5 cell and single hESC samples. Samples were obtained from two independent, in-house derived, primed hESC lines and their naive counterparts. The direct comparison of genomic profiles validated our approach and allowed for CNV detection at high resolution.
Participants/materials, setting, methods: Samples were collected from primed hESC cultures at time of passage, with remaining cells seeded into our established naive conditions. Subsequently, naive hESC samples were collected at passage 2 during conversion and every second passage thereafter, for at least 10 passages. Whole genome amplification was performed only on single cell and 3–5 cell samples, prior to library preparation. Shallow whole genome sequencing was performed on an Ion Proton instrument using 400 flows.
Main results and the role of chance: A number of unique Mb scale structural variants were detected in a high proportion of cells in both primed and naive bulk samples. The first primed hESC line carried a trisomy 17 and gains of 11q23.1 and 20q13.2. These aberrations were also present in some, but not all, 3–5 and single cell samples. They further persisted in naive hESC cultures across all passages analysed. For the same cell line, a further trisomy 3 was observed in one of the naive single cell samples. In the second primed hESC line we detected an amplification of 1q32.1 in most samples. In the respective naive line, this gain seemed to be present in a higher fraction of the bulk cells. The 20q13.2 amplification was the smallest called CNV at 4 Mb. This served to define our sequencing resolution and exceeded those previously reported for whole-genome single cell hESC microarrays.
Limitations, reasons for caution: To thoroughly investigate the genomic effects of naive culture, expanding our study to incorporate further cell lines across lower and higher passages is necessary. The known bottlenecks of whole-genome single cell sequencing, such as uneven genome fragmentation and amplification, as well as inability to verify results, warrant careful interpretation.
Wider implications of the findings: High-resolution single cell whole-genome analysis holds unprecedented potential for evaluating the genetic diversity of hESCs. Investigating their heterogeneity, particularly during cellular transformations, may help unravel driving mechanisms of genomic instability. Ultimately, this could facilitate safer clinical implementation of hESCs.
Trial registration number: N/A.

O-181 Human menstrual blood-derived mesenchymal stem cells repair injured endometrium by angiogenesis *via* activating AKT and ERK pathways

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Study question: Do human menstrual blood-derived mesenchymal stem cells (MB-MSCs) play a role in repairing injured endometrium?
Summary answer**:** Endometrial thickness and mice fertility were improved after MB-MSCs transplant in endometrium injury mice, probably by promoting endometrial angiogenesis.
What is known already: Intrauterine adhesion (IUA) is a common acquired endometrial disorder secondary to endometrium injury such as trauma, curettage and infection. Ischemia is thought to be the major insult leading to the development of postoperative intra-abdominal and pelvic adhesions such as peritoneal adhesion and IUA. Stem cell-based therapy has shown prospective therapeutic effects on ischemic disease and tissue regeneration.
Study design, size, duration: Endometrium injury model were established with ICR mice ($n = 106$). In MB-MSCs group, MB-MSCs were transplanted by tail vein injection. In control group, normal saline was injected. Uterine were collected at Day 7 ($n = 17$ for MB-MSCs group, $n = 16$ for control group) and Day 14 ($n = 13$ for MB-MSCs group, $n = 11$ for control group) after MB-MSCs transplant for the histopathological observations. Seven days after MB-MSCs transplantation, female mice were mated with male mice ($n = 28$ for MB-MSCs group, $n = 14$ for control group).
Participants/materials, setting, methods: *In vivo*, we evaluate endometrial thickness, microvessel density and fertility function in mice model after MB-MSCs transplantation. *In vitro*, MB-MSCs conditioned medium (MB-MSCs CM) were collected and HUVECs were treated by MB-MSCs CM. Proliferation, apoptosis, migration and angiogenesis assay in HUVECs were performed and phosphorylated and total AKT, p38, JNK, ERK levels in HUVECs were determined by western blot. Furthermore, qPCR was applied to determine transcriptional level of downstream angiogenesis-related genes of AKT and ERK.
Main results and the role of chance: *In vivo*, we could find that Dil-labeled MB-MSCs engraf in the injured endometrium at Day 7 and Day 14 by small animal imaging. Seven days after transplantation, endometrial thickness and microvessel density presented a significant increase after MB-MSCs transplantation compared with that of control group ($p = 0.002$ for thickness, $p = 0.007$ for MVD respectively). Conception occurred in all the undamaged horns. In MB-MSCs group 15 of 28 damaged horns conceived, whereas only 2 of 14 in control group conceived ($p = 0.014$, *Chi*-square). The embryo number from the damaged horns in MB-MSCs group were obviously higher than control group (3.1 ± 0.6 vs. 0.9 ± 0.7, $p = 0.030$). *In vitro*, MB-MSCs CM promoted HUVECs growth and migration, while decreased apoptosis. Formation of vascular-like

networks was also remarkably enhanced after MB-MSCs CM treatment. Quantitative analysis of loop numbers, tube length, and vessel area showed that MB-MSCs CM improved HUVECs angiogenesis ($p < 0.05$). For mechanism, AKT and ERK phosphorylation levels in HUVECs increased after MB-MSCs CM treatment. Furthermore, qPCR showed that expressions of *eNOS*, *VEGFA*, *VEGFR1*, *VEGFR2*, and *TIE2* which are downstream genes of AKT and ERK in HUVECs were increased after MB-MSCs CM treatment.
Limitations, reasons for caution: MB-MSCs CM may secrete many factors favoring angiogenesis, immunity, and inflammation. However, factors that really works in our experiment was not clear. Additional work is needed to look for these factors.
Wider implications of the findings: MB-MSCs restore injured endometrium and improve mice fertility, which is promoted by angiogenetic effect of MB-MSCs, indicating its potential application in uterine repair and IUA prevention.
Trial registration number: No.

O-182 Detection, isolation and characterization of differentiation-resistant human embryonic stem cells

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Study question: Can we quantify, isolate and characterize differentiation-resistant human embryonic stem cells to investigate the mechanisms that lay at the origin of their differentiation deficiency?
Summary answer: Residual undifferentiated stem cells (rSC) can be detected down to the level of 0.02%, isolated from differentiated populations and further propagated for characterization.
What is known already: Often during differentiation of human embryonic stem cells (hESC), rSC that apparently have lost differentiation capacity are observed. The transplantation of differentiated cell populations containing these cells may pose a high risk of tumor formation in patients and therefore represent a major hurdle to exploit the full therapeutic potential of hPSC. However, very little research on the mechanisms by which cells remain undifferentiated is currently available.
Study design, size, duration: Three undifferentiated hESC lines (VUB02, VUB03_DM1 and VUB14) and their differentiated subpopulations were used in this study. Cells were let to spontaneously differentiate by applying medium containing serum, while omitting bFGF supplementation. Cultures were considered fully differentiated after 21 days.
Participants/materials, setting, methods: We have developed detection methods utilizing a variety of techniques, including flow cytometry, immunofluorescence, a colony formation assay and real-time PCR. Line specific standard curves were generated by spiking of defined numbers of undifferentiated cells in fibroblast populations. Isolation of rSC was achieved through the use of a novel replating technique, whereby small amounts of a differentiated population were re-introduced into culture conditions that favor undifferentiated hPSC proliferation.
Main results and the role of chance: While we readily detected rSCs within differentiated populations, detection ranged from 0.023 to 8.1%, depending on the line under investigation and the applied detection technique. Several possible sources of error in our estimates make the exact quantity of rSC difficult to evaluate. Nonetheless, ranking of the three lines based on the number of rSC detected was identical for the different techniques applied.

The plating technique allowed for the clonal growth of rSC colonies that could easily be isolated. Residual SC lines were isolated and propagated from two out of three hESC lines after 21 days of differentiation. Of these two, one line was karyotypically normal prior to the start of differentiation. The rSC line obtained, however, was abnormal and displayed a chr20p deletion and chr20q amplification, suggesting a possible link between copy number variations and the occurrence of rSC.
Limitations, reasons for caution: Although a well-characterized mutation at 20q11.21 has been shown to give hESC a significant growth advantage, due to the size of the observed mutation, we cannot confirm whether the same driver gene is involved in the change of differentiation potential.
Wider implications of the findings: We demonstrate the high occurrence of rSC in differentiated populations, a possible safety risk in the clinical application of hPSC-derived products. Our rSC isolation method, however, can be an important tool for further research into the mechanisms causing rSC and for the unraveling of pluripotency mechanisms.
Trial registration number: N/A.

O-183 Opposing effects of FGF2 and TGF-β/Activin/Nodal signalling inhibition on BMP-mediated trophoblast differentiation in hESCs

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Study question: What is the effect of inhibiting fibroblast growth factor 2 (FGF2) and transforming growth factor beta (TGF-β)/Activin/Nodal signalling in bone morphogenic protein 4 (BMP4)-induced trophoblast differentiation?
Summary answer: Inhibition of FGF2 and TGF-β/Activin/Nodal signalling had opposing effects on BMP4-mediated differentiation of human embryonic stem cells (hESCs) into trophoblast-like cells.
What is known already: Several studies have demonstrated that hESCs can be differentiated into trophoblast-like cells if exposed to BMP4 and/or inhibitors of FGF2 and the TGF-b/Activin/Nodal signalling pathways. However, how much the inhibitors of these pathways can improve the efficiency of hESC differentiation compared to basic BMP4 treatment, and the individual effects and synergy between the inhibitors, remain unknown.
Study design, size, duration: Full transcriptome analysis by RNA sequencing was used to analyse the effects of inhibitor combinations on the differentiation of the hESC line H9 into trophoblast-like cells over 12 days. Two biological replicates for four different conditions were analysed over seven time points.
Participants/materials, setting, methods: For each time point, genes differentially expressed to those in untreated cells were identified by the edgeR package. Additionally, expression of total chorionic gonadotropin (hCG) and its hyperglycosylated form (hCG-H) were determined by immunoassay from culture media.
Main results and the role of chance: FGF2 inhibition directs hESCs toward a similarity with placental tissue, up-regulates syncytiotrophoblast-specific genes (*CGA*, *CGB*, *CYP11A1* and *HOPX*) and induces several molecular pathways involved in embryo implantation. In addition, we demonstrated that the inhibition of FGF2 is the key trigger of hCG-H production. In contrast, inhibition of the TGF-β/Activin/Nodal pathway decreases the ability of hESCs to form trophoblast-like cells.
Limitations, reasons for caution: In this study, the hESC line H9 was used as an *in vitro* model system. The effects of inhibited FGF2 and/or TGF-β/Activin/Nodal signalling pathways were exclusively assessed *via* cell differentiation. Other potentially relevant pathways were not investigated.
Wider implications of the findings: A better understanding of the key players in the differentiation of trophoblast cells from hESCs will help us to develop an optimal model for studying the early development of human trophoblasts in normal pregnancy and pregnancy complications.
Trial registration number: This study is basic study.

SELECTED ORAL COMMUNICATIONS

SESSION 50: EFFECTS OF AGE AND HEALTH ON SEMEN PARAMETERS AND SPERM DNA INTEGRITY

Tuesday 05 July 2016 **Room 101** **15:15–16:30**

O-184 A comprehensive study into the effects of advancing male age on semen parameters, sperm genetic integrity and the outcome of assisted reproductive treatments

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Study question: What is the impact of advancing male age on conventional semen parameters, sperm DNA integrity and aneuploidy, fertilisation, embryo development and pregnancy?
Summary answer: Based on this study advanced paternal age can have an adverse effect on sperm DNA integrity and the outcome of assisted reproductive treatments.
What is known already: Numerous investigations have demonstrated the influence of female age on fertility and IVF outcomes. However, the role of male age has received far less attention and remains relatively poorly understood. Some studies suggest that natural fertility declines with advancing male age. Others have demonstrated a correlation between elevated paternal age and incidence of certain genetic disorders. Furthermore, increased sperm DNA fragmentation with age has also been reported. Despite this data, the impact of male age on fertility is considered controversial and requires further investigation. We sort to shed light on this question through a large study examining multiple semen parameters.
Study design, size, duration: This was a retrospective study of 590 men from infertile couples undergoing assisted reproduction techniques. Data was available corresponding to fourteen parameters, which were descriptive of semen quality, sperm function and treatment outcome. Together, this data allowed a comprehensive review of the impact of age on male reproductive function. Additionally, relationships between individual parameter, independent of age, were also evaluated.
Participants/materials, setting, methods: Factors considered with respect to paternal age included multiple traditional sperm parameters, sperm DNA integrity, sperm aneuploidy rate, fertilization rate and 2PN cleaved embryo rate and live birth rate. Life-style factors and prior medical history were also noted. DNA fragmentation analysis involved the Sperm Chromatin Dispersion (SCD) test, while cytogenetic analysis utilised fluorescence in situ hybridisation (FISH) for five chromosomes in >2,000 sperm. Statistical evaluation was performed using correlation (Pearson test) and linear regression.
Main results and the role of chance: Analysis of the data revealed that most traditional sperm parameters, such as concentration and sperm abnormality were not affected by paternal age. The only exception being motility, which was significantly reduced with paternal age (partners aged more than 35 were excluded) [$F(1, 97) = 7.98$, $p < 0.05$, $R^2 = 0.076$]. Linear regression analysis also showed that advanced male age was associated with increasing sperm DNA fragmentation (SDF) [$F(1, 551) = 4.556$, $p < 0.05$, $R^2 = 0.008$] and elevated DNA degradation [$F(1, 570) = 23.49$, $p < 0.001$, $R^2 = 0.04$]. Regardless of age, alcohol intake was observed to adversely affect SDF rates [$F(1, 104) = 5.685$, $p < 0.05$, $R^2 = 0.052$]. Furthermore, increased male age was associated with a decrease in the average number of 2PN cleaved embryos ($p < 0.05$), fertilization rate ($p < 0.05$) and ultimate birth rate ($p < 0.001$). Advance paternal age and alcohol consumption in male patient independently elevated the SDF and SDFI and subsequently alteration in these two factors disclosed their negative effects on the number of 2PN cleaved embryos form IVF cycles ($p < 0.05$).
Limitations, reasons for caution: A potential limitation of this study is that we did not have access to a fertile population. A great proportion of the data was based on subfertile patients attending an IVF clinic and therefore might not be representative of the actual male population.
Wider implications of the findings: A negative impact of male age on assisted reproductive treatments was confirmed, leading to decreased birth rates. Results obtained provide evidence that factors such as sperm DNA damage may contribute to this age-effect, suggesting that measuring this parameter, and attempting to mitigate elevated levels, may be valuable for older patients.
Trial registration number: N/A.

O-185 The effects of age on sperm DNA damage: an evaluation of 2,178 semen samples

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Study question: What are the effects of aging on sperm DNA damage?
Summary answer: Sperm DNA fragmentation worsened with age and seems to be associated with mitochondrial damage. The observed increase in protamination with age needs further analysis.
What is known already: Evidence suggests that there are declines in semen quality and male fertility associated with increasing male age. Advanced paternal age has been implicated in the increase in the frequencies of abortions, autosomal dominant diseases, aneuploidy, and other diseases. Advanced male age has also been correlated with infant mortality. One plausible explanation for these results is that older men may have more sperm with damaged DNA. Chromatin damage has been associated with male infertility, conception problems, and difficulty sustaining pregnancy. There is also evidence linking DNA damage in sperm with the risk of mutations and birth defects in the offspring.
Study design, size, duration: A cross-sectional study of semen samples obtained from 2,178 men (37.9 ± 6.4 years) from couples who attended an infertility clinic was conducted. The semen analysis results from one semen sample from each man were recorded. For DNA integrity analysis, the following percentages were determined:

- Spermatozoa with DNA fragmentation using the TUNEL assay;
- Spermatozoa with abnormal chromatin packaging/underprotamination using chromomycin A3 (CMA3);
- Spermatozoa with abnormal mitochondrial membrane potential (MMP) using MitoTracker Green;
- Spermatozoa in apoptosis using annexin-V.

Participants/materials, setting, methods: For two-group comparisons, the subjects were categorized according to age into three groups: ≤35 years, 36–44 years and ≥45 years. Associations between age and sperm parameters were assessed using Spearman correlation. At least 200 spermatozoa were examined in each evaluation. Potential confounders (abstinence time, smoking, alcohol, and varicocele) were also observed. A sample size of 300 subjects in each group has 80% power (alpha 0.05) to detect a difference of 15% between groups.
Main results and the role of chance: Sperm DNA damage seems to be influenced by the aging process. Although the influence of aging on sperm apoptosis was not observed, sperm DNA fragmentation and MMP (mitochondrial damage) worsened with age. However, chromatin packaging (protamination) increased with age. Tables 1 and 2 show the results.

No correlations between abstinence, smoking, alcohol, and varicocele were shown.

Table 1. Men's age × semen parameters: Spearman's correlation.

Outcomes	Correlation coefficient	*p*
DNA fragmentation (%)	0.10	0.002
Abnormal MMP (%)	0.14	<0.0001
Apoptosis (%)	0.03	0.28
CMA3 positivity (%)	−0.13	<0.0001

Table 2. Men's age × semen parameters: group comparisons. Values within rows with the same superscript letter were significantly different.

Outcomes	≤35	36–44	≥45	*p*
n	852	1014	312	
DNA fragmentation (%)	14.7 ± 8.3[a,b]	15.9 ± 8.7[a]	16.1 ± 8.4[b]	0.002[a], 0.009[b]
Abnormal MMP (%)	24.6 ± 16.4[a]	25.6 ± 16.0[b]	29.0 ± 17.1[a,b]	0.006[a], 0.04[b]
Apoptosis (%)	19.1 ± 8.0	19.3 ± 7.9	19.3 ± 7.8	0.85
CMA3 positivity (%)	57.7 ± 15.0[a,b]	55.7 ± 15.1[a,c]	52.9 ± 15.6[b,c]	0.01[a], <0.0001[b], 0.01[c]

Limitations, reasons for caution: These data are cross-sectional and specifically focus on the association between age and DNA damage. Only men from infertile couples were sampled.
Wider implications of the findings: The age-related increase in sperm DNA damage suggests that delaying childbearing, not only in women but also in men, may jeopardise reproductive capacity. The increase in chromatin packaging may

represent a protective feature for DNA. Additional studies should be performed to confirm the results concerning chromatin packaging (chromatin protamination) in sperm.
Trial registration number: Not applicable. The local ethics committee authorised this study.

O-186 Semen parameters in men with varicocele: DNA fragmentation, chromatin packaging, mitochondrial membrane potential, and apoptosis

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Study question: Does varicocele affect the sperm DNA integrity?
Summary answer: Individuals with varicocele show increased DNA damage associated with an increase in abnormal mitochondrial activity and an increase in abnormal chromatin packaging (underprotamination).
What is known already: Sperm DNA integrity is considered a marker of spermatogenesis integrity and male fertility potential. High levels of sperm DNA damage have been significantly linked to lower rates of spontaneous conception and assisted reproductive pregnancies. Varicocele have been associated with increased levels of reactive oxygen species and decreased seminal antioxidant capacity, increased sperm DNA damage, and defective spermatogenesis in affected patients. Although there is literature examining the possible effects of varicocele on semen DNA, the sample sizes in several studies are insufficient to generate significant results.
Study design, size, duration: A cross-sectional study was conducted with semen samples from 2,399 men randomly selected from couples who attended an infertility clinic. A total of 16.3% (391/2,399) of the men had varicocele. A physical examination and ultrasound were used to diagnose varicocele. Statistical analysis was performed using logistic regression.
Participants/materials, setting, methods: In each semen sample, which was collected after sexual abstinence of 2–5 days, at least 200 spermatozoa were examined (in each evaluation), and the percentages of the following parameters were measured:

- Spermatozoa with DNA fragmentation using the TUNEL assay.
- Abnormal chromatin packaging using chromomycin A3 (CMA3).
- Abnormal mitochondrial membrane potential (MMP) using MitoTracker Green.
- Apoptosis using annexin-V.

Samples were also evaluated according to WHO guidelines/morphology-motile sperm organelle morphology examination/MSOME. Potential confounder parameters (age, abstinence, BMI, smoking, and alcohol) were also observed.
Main results and the role of chance: Regression analysis revealed that the percentages of TUNEL-positive sperm, CMA3-positive sperm, and abnormal MMP sperm were significantly increased in individuals with varicocele compared to men without varicocele. Apoptosis was not influenced by varicocele. General semen parameters were significantly worse in individuals with varicocele. Table 1 shows the data.

Table 1. Logistic regression: varicocele and semen parameters.

	Varicocele		Logistic regression		
	Yes	No	Odds ratio	95% Confidence Interval	*p*
n	391	2008			
Age (years)	37.7 ± 6.3	37.8 ± 6.6	1.00	0.98–1.01	0.97
BMI (kg/m²)	28.3 ± 4.4	28.1 ± 19.8	0.99	0.95–1.02	0.35
Smoking/habit (%)	10.5	11.9	0.86	0.61–1.23	0.41
Alcohol/intake (%)	66.2	67.0	0.97	0.77–1.22	0.77
DNA Fragmentation (%)	16.3 ± 8.8	15.3 ± 8.5	1.02	1.01–1.03	0.03
Apoptosis (%)	18.8 ± 7.4	19.2 ± 8.1	0.99	0.97–1.01	0.45
CMA3 positive (%)	58.9 ± 14.8	55.6 ± 15.2	1.02	1.01–1.03	0.001
Abnormal MMP (%)	28.3 ± 16.9	25.4 ± 16.2	1.02	1.01–1.03	0.03
Normal forms (%)	0.4 ± 0.9	0.9 ± 1.5	0.70	0.61–0.81	<0.0001
Concentration (×10⁶/ml)	44.4 ± 45.5	76.4 ± 57.2	0.98	0.97–0.99	<0.0001
Progressive motility (*n*)	51.2 ± 17.6	57.0 ± 16.5	0.98	0.97–0.99	<0.0001
Total motility (*n*)	59.1 ± 17.2	63.7 ± 16.0	0.98	0.97–0.99	<0.0001
Leukocytes (×10⁶/ml)	0.4 ± 1.4	0.7 ± 0.8	1.06	0.96–1.16	0.22
Vitality (%)	60.3 ± 16.3	65.3 ± 15.7	0.98	0.97–0.98	<0.0001
pH	8.1 ± 0.4	8.0 ± 0.4	1.4	1.03–1.82	0.02
Volume (ml)	2.5 ± 1.1	2.8 ± 1.6	0.90	0.82–0.99	0.02
Abstinence (days)	3.6 ± 9.1	3.6 ± 2.7	1.00	0.98–1.02	0.84

Limitations, reasons for caution: The sperm were obtained from men who underwent fertility evaluations. Generalisation of these results to the general population should be performed with caution.
Wider implications of the findings: The presence of varicocele may impair sperm DNA integrity and fertility. Given the extant literature, varicocele repair and antioxidant intake should be considered to reduce DNA damage in sperm.
Trial registration number: Not applicable. The local ethics committee authorised the study.

O-187 Association between body mass index (BMI) and sperm quality or sperm DNA integrity. A large population study

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Study question: Is overweight associated with a decrease in sperm quality or impaired sperm DNA integrity?
Summary answer: High BMI negatively impacts sperm quality. High BMI is not associated with sperm DNA fragmentation, apoptosis, or protamination but is associated with increased mitochondrial damage.
What is known already: Obesity appears to influence male infertility and male reproductive dysfunction negatively. Overweight and obesity have been associated with significant reductions in the levels of total testosterone, free testosterone, and sex hormone-binding globulin and with elevated levels of estrogens. In addition, the accumulation of suprapubic and inner fat may cause scrotal hyperthermia, which is a probable cause of elevated oxidative stress. Some studies have highlighted impairments of sperm quality for patients with BMI, but the results are conflicting. Sperm DNA integrity is another factor that may be affected in obese men; however, previous studies have shown controversial results regarding this influence.
Study design, size, duration: A cohort of 1,824 men from couples who underwent infertility evaluations was recruited, and BMI measurements were made at this time. Pathological conditions of the genital tract/azoospermia were exclusion criteria. The semen analysis results from one semen sample from each man were recorded (WHO criteria/morphology-motile sperm organelle morphology examination/MSOME). For DNA integrity analysis, the percentages of DNA fragmentation (by TUNEL assay), abnormal chromatin packaging/underprotamination (by chromomycin A3/CMA3), abnormal mitochondrial membrane potential (MMP/by MitoTracker Green), and apoptosis (by annexin-V) were recorded.
Participants/materials, setting, methods: For two-group comparisons, the subjects were categorized according to BMI into three groups: ≤24.9, 25.0–29.9 (overweight) and ≥30.0 kg/m² (obese). Associations between BMI and these outcomes were assessed using Spearman correlation. At least 200 spermatozoa were examined in each evaluation. Potential confounders (age, abstinence time, smoking, alcohol, and varicocele) were also observed. A sample size of 250 subjects in each group has 80% power (alpha 0.05) to detect a difference of 15% between groups.
Main results and the role of chance: No correlations between BMI and age, abstinence, smoking, alcohol, and varicocele were observed. The regression analysis showed significant decreases in the sperm concentration, motility,

normal morphology, and vitality with increasing male BMI. Conversely, there was a significant positive correlation between the percentage of spermatozoa with abnormal MMP (mitochondrial damage) and BMI (Table 1). Similarly, in two-group comparisons, sperm concentration, progressive motility, morphology, vitality, and mitochondrial membrane potential worsened with the increase of BMI (Table 2).

Table 1. BMI × semen parameters: Spearman's correlation.

Outcomes	Correlation coefficient	*p*
pH	0.04	0.10
Volume (ml)	0.02	0.34
Concentration (ml × 10^6)	−0.18	<0.0001
Progressive motility (%)	−0.19	<0.0001
Normal forms (%)	0.11	<0.0001
Leukocytes (×10^6/ml)	0.03	0.13
Vitality (%)	0.17	<0.0001
DNA fragmentation (%)	0.01	0.76
Apoptosis (%)	0.01	0.81
CMA3 positivity (%)	0.02	0.37
Abnormal MMP (%)	0.24	<0.0001

Table 2. BMI × semen parameters: group comparisons.

Outcomes	BMI: ≤24.9 kg/m²	BMI: 25–29.9 kg/m²	BMI: ≥30 kg/m²	*p*
n	370	856	598	
pH	8.0 ± 0.3	8.0 ± 0.3	8.1 ± 0.4	0.94
Volume (ml)	2.8 ± 1.5	2.9 ± 1.6	2.8 ± 1.6	0.53
Concentration (ml × 10^6)	83.5 ± 62[a]	77.1 ± 56.3[b]	58.8 ± 51.1[a,b]	<0.0001[a,b]
Progressive motility (%)	58.6 ± 13.8[a]	56.8 ± 14.6[b]	51.5 ± 17.5[a,b]	<0.0001[a,b]
Normal forms (%)	0.7 ± 0.9[a]	0.6 ± 0.8[b]	0.4 ± 0.7[a,b]	<0.0001[a,b]
Leukocytes (×10^6/ml)	0.3 ± 0.6	0.4 ± 0.9	0.4 ± 1.1	0.66
Vitality (%)	67.6 ± 12.3[a,b]	65.0 ± 13.6[a,c]	60.8 ± 15.6[b,c]	0.009[a], <0.0001[b,c]
DNA fragmentation (%)	14.4 ± 7.5	14.5 ± 8.2	14.5 ± 7.4	0.74
Apoptosis (%)	19.5 ± 8.1	19.2 ± 8.0	19.1 ± 7.6	0.75
CMA3 positivity (%)	56.3 ± 15.5	55.7 ± 15.1	56.4 ± 15.1	0.73
Abnormal MMP (%)	20.6 ± 13.1[a,b]	25.3 ± 15.7[a,c]	30.9 ± 18.3[b,c]	0.0004[a], <0.0001[b], 0.0001[c]

Limitations, reasons for caution: A limitation might be the cross-sectional nature of the data. Furthermore, this study was conducted in couples who sought fertility treatment and could therefore be biased toward infertility. The generalisation of these results to the general population should be performed with caution.

Wider implications of the findings: Given the adverse consequences of obesity, the benefits of weight reduction should be discussed when counselling couples interested in fertility treatment. The exact mechanisms that mediate the effects of obesity on the mitochondrial damage of sperm require additional investigation.

Trial registration number: Not applicable. The local ethics committee authorised this study.

O-188 Prevalence of human papilloma virus in sperm, Sperm DNA Fragmentation Index (DFI %) and ART success in couples with repeated implantation failure

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Study question: What is the sperm HPV prevalence in males with RIF history and potential impact on sperm parameters, sperm DNA fragmentation index (DFI) and reproductive outcomes in couples with RIF?

Summary answer: Overall HPV prevalence was 7.7% (9/119) with a mean DFI of 39.1%. Semen parameters and implantation rates were similar between those with or without HPV.

What is known already: HPV infection is associated with reduced sperm motility contributing to male infertility. Moreover, HPV infected spermatozoa was also linked with increased miscarriages.

Study design, size, duration: A total of 117 male partners of couples with RIF history undergoing ART were included in this cross-sectional study between 2014 and 2015.

One ART cycle of each patient was included in the study.

Demographic characteristics, hormone profiles, ART cycle outcomes, semen parameters, sperm DFI (%) and reproductive outcomes were compared among those with or without HPV in spermatozoa.

Participants/materials, setting, methods: A total of 117 couples with RIF history were enrolled to the study in a single ART centre.

The seminal plasma was used for detection of HPV by a commercially available kit.

TUNNEL assay was performed to detect DFI by commercially available kit. In each sample 300 spermatozoa was counted at fluorescent microscope.

Ovarian stimulation was carried out using antagonist protocol.

Semen parameters, DFI and reproductive outcomes were compared among those with or without HPV DNA.

Main results and the role of chance: Overall sperm-HPV rate was 7.7% (9/119) in male subjects of couples with RIF history.

Demographic characteristics and basal hormonal profiles were similar among those with or without HPV in spermatozoa.

Semen parameters including volume, count and motility were similar among those with or without HPV in spermatozoa.

Overall Sperm DFI was detected as 39.1% and was found to be similar between the two groups (37.6 vs. 38.3% in HPV and HPV groups respectively, $p > 0.05$).

Fertilization (82.5 vs. 76.5%), implantation (29.1 vs. 33.3%) and ongoing pregnancy rates (11.1 vs. 28.7%) were comparable among groups without significant difference. Relatively higher miscarriages were detected in HPV+ group without reaching a statistically significant difference (33.3 vs. 10.2%).

Limitations, reasons for caution: Relatively small sample size of our cohort.

Further studies are needed to confirm the correlation between HPV male infection and reproductive outcomes of couples with repeated ART failures.

Wider implications of the findings: Overall HPV prevalence of 7.7% is in accordance with the previously reported prevalence (7, 8–11%).

Also, HPV infection did not seem to affect semen quality.

HPV infection of men can be another factor for lower ongoing pregnancy rates and higher miscarriages in couples with high sperm DFI and RIF history.

Trial registration number: ACTRN12614000188639.

SELECTED ORAL COMMUNICATIONS

SESSION 51: OOCYTE AND SPERM QUALITY

Tuesday 05 July 2016 **Hall 1** **17:00–18:00**

O-189 Follicular size is a confounding factor for gene expression analysis in cumulus cells

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Study question: Does follicular size have an influence on the level of cumulus cell gene expression in embryos that develop to blastocyst or arrest during development?

Summary answer: Gene expression varies in developing blastocysts according to follicular size and between arrested embryos and those developing to blastocyst.

What is known already: Many studies have been performed on cumulus gene expression with the aim of correlating gene expression levels with different outcome measures. However, the results were not consistent between different studies. Although the field has been extensively investigated, no predictive marker has been identified so far. A possible explanation is the rather small number of COC samples that was available in these studies. Another aspect that has not been studied so far is the potential effect of confounding factors that may have an influence on cumulus gene expression.
Study design, size, duration: This study was performed between July 2014 and September 2015. Strict inclusion (<2 previous treatment cycles, age ≤39 years, ≥8 oocytes retrieved) and exclusion criteria (PGD or PGS indication, >24 COC during pickup) were applied. Oocytes were injected and cultured to blastocyst stage at 6% CO_2 and 5% O_2 using a single-step culture medium (Global) with change on day 3. Cumulus cells were collected from a total of 2560 COC for further subgroup analysis.
Participants/materials, setting, methods: Cumulus gene expression was studied in small (<17 mm on OPU day; $n = 699$) vs. large ($n = 788$) follicles and in embryos that arrested ($n = 682$) or reached blastocyst on day 5 ($n = 805$). We used 5 genes for qRT-PCR analysis of RNA expression levels (Lightcycler 480). In addition 41 cumulus samples representing embryos from small and large follicles that developed to blastocysts or arrested before day 3 were analyzed by whole genome expression analysis (Affymetrix Genechip Human Gene 2.0ST Array).
Main results and the role of chance: We first applied qRT-PCR to analyze well-described genes that are expressed in cumulus cells (HAS2, PTSG2, PTX3, TNFAIP6 and GDF9). When compared to arrested embryos, embryos that developed to blastocysts showed a significant downregulation for HAS2, PTSG2 and TNAFIP6 ($p < 0.05$). Looking at follicular origin, HAS2 and PTSG2 were downregulated for all embryos from small compared to large follicles ($p < 0.0005$). When we combined embryo development and follicular origin, only HAS2 was downregulated in blastocysts from small compared to large follicles ($p < 0.05$). However, we also noted a downregulation of HAS2 and PTSG2 in arrested embryos from small follicles compared to those reaching blastocyst stage ($p < 0.0005$). These fluctuations reflect the complexity of translating such a basic scientific study from bench to bedside.

The results from genome expression were subjected to a principal component analysis to allow for an effective interpretation. Embryos that arrested during development showed similar gene expression patterns independent of follicle size. Embryos that developed to blastocysts showed clear differences to arrested embryos but also differences between large and small follicles. This was reflected by ANOVA analysis showing significance for differentially expressed transcripts. These results may reflect the heterogeneity of the follicular origin of human embryos.
Limitations, reasons for caution: Results were obtained with a limited number of genes studied and cannot automatically be translated to other potential candidate genes. For the second part of the study results were obtained from a small number of samples, which may not necessarily be representative for a larger cohort.
Wider implications of the findings: Cumulus gene expression studies are affected by several confounding factors that are based on follicular size and development, which may also reflect the heterogeneity of patient population undergoing IVF. Such factors should be taken into account when searching for predictive markers for IVF outcome.
Trial registration number: Not applicable.

O-190 Correlation between oocyte cumulus cells gene expression, embryo morphokinetic, blastocyst formation and euploidy

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Study question: Is cumulus cells (CC) gene expression pattern of five candidate genes, combined with morphokinetic parameters a non-invasive method to predict blastocyst formation and euploidy?
Summary answer: CCs gene expression is correlated to embryo development and morphokinetic behaviour. Among blastocysts, we found PTGS2 over-expressed in CCs of euploid embryos.
What is known already: Due to the crosstalk within the follicle, CC gene expression has been proposed as a non-invasive tool to predict oocyte/embryo competence. New promises come also from time-lapse, however no clear agreements on which gene and/or morphokinetic parameter can be used to predict embryo quality. A critical aspect of embryo quality is the blastocyst chromosomal status, and this feature can be used as an outcome measure to test potential biomarkers of oocyte quality.
Study design, size, duration: Prospective longitudinal cohort study (September 2014–September 2015). A total of 58 CCs samples were individually collected from 21 consenting patients. Oocytes and belonging embryos where cultured individually in a time-lapse incubator (Embryoscope), until blastocyst stage. 29 oocytes (from 16 patients) reached blastocyst stage and underwent comprehensive chromosomal screening. Collected CCs samples were analysed for the expression of 5 candidate genes, previously found as predictive of oocyte competence: HAS2, PTGS2, CAMK1D, EFNB2, and STC1.
Participants/materials, setting, methods: Advanced maternal age patients (38.3 ± 2.5 years), underwent ICSI and pre-implantation genetic testing for aneuploidy. Severe male factor was excluded. All ovarian stimulations were performed with a fixed protocol. Retrieved cumulus-oocyte complexes were denuded individually. CCs gene expression was performed through qPCR using Taqman gene expression assays (Applied Biosystems). Twelve morphokinetic parameters were recorded during embryo culture. T-tests and Spearman's rank correlation were performed through SigmaPlot (SSI, CA, USA).
Main results and the role of chance: The 5 selected genes are known to be involved in cumulus expansion [hyaluronan acid synthase 2 (HAS2) and prostaglandin-endoperoxide synthase 2 (PTGS2)], in the mediation of developmental events [EphrinB2 (EFNB2) Ca-/calmodulin–dependent protein kinase 1 (CAMK1D)] and in autocrine and paracrine functions [Stanniocalcin-1 (STC1)]. Analysing CC gene expression of blastocysts versus arrested embryos, blastocyst formation was negatively correlated with EFNB2 expression ($p = 0.035$; 95% CI: 0.0553–1.463. Logistic regression model adjusted for female age confirmed this association, OR = 2.7, 95% CI = 1.1–7.3.

Combined analysis of morphokinetics and gene expression showed a negative correlation between timings of singamy and of second cell cycle and STC1 expression ($p < 0.004$) while timings of 4-cell divisions were related to HAS2 expression ($p = 0.032$). EFNB2, PTGS2 and CAMK1D expressions showed a positive correlation to timings of starting blastulation ($p = 0.015$).

Among obtained blastocysts we analyzed CC gene expression patterns of 18 aneuploid versus 11 euploid blastocysts, finding PTGS2 over-expression ($p = 0.026$; 95% CI: 0.180–2.515) in euploid blastocysts.
Limitations, reasons for caution: This study is limited to a specific population of patients, a relatively low number of CCs samples and a restricted number of genes. Results must be confirmed on larger scale
Wider implications of the findings: We found a correlation between CC gene expression of 5 selected genes, morphokinetics and blastocyst formation while euploidy could only be related to PTGS2 expression. We confirmed the potentiality of CC gene expression analysis in predicting embryo competence.
Trial registration number: None.

O-191 Sperm DNA integrity is associated with time-lapse parameters of early embryonic development

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Study question: Is there any interaction between DFI and fertilization method in relation to early embryo development?
Summary answer: An association between the level of impairment of sperm DNA integrity and early embryo development parameters as assessed by time-lapse are demonstrated.
What is known already: We recently demonstrated that high sperm DNA fragmentation index (DFI) is associated with a lower fertilization rate in standard IVF but not in ICSI. Therefore, by time-lapse microscopy, we studied the interaction between DFI and fertilization method in relation to early embryo development

Study design, size, duration: This is a retrospective study based on 6117 oocytes from 256 IVF and 383 ICSI treatments performed at the Reproductive Medicine Centre, Skåne University Hospital, Malmö, Sweden.
Participants/materials, setting, methods: The DFI values were categorized into 3 intervals: DFI ≤ 10% (reference group), 10% < DFI ≤ 20%, and DFI > 20%. Endpoints were meantime of formation of pronuclei (tPNa), meantime of fading of pronuclei (tPNf), meantime of early cleavage (t2) and meantime of starting blastulation (tSB). Data were analyzed using univariate analysis of variance in three ways; the interaction between DFI category and fertilization type, separately for IVF and for ICSI and also ICSI compared to standard IVF.
Main results and the role of chance: In the ICSI group the meantime of tPNa was significantly lower for 10% < DFI ≤ 20% and DFI > 20, as compared to the reference group (DFI ≤ 10%). The meantime of tPNf increased statistically significant in the standard IVF group for 10% < DFI ≤ 20% and DFI > 20 as compared to the reference group. When comparing ICSI to IVF, the mean t2 time was statistically significantly higher for the latter if DFI was above 20%. The meantime of starting blastulation was significantly longer in the ICSI group for DFI ≤ 20 as compared to the reference group, but no such association was observed within the IVF/ICSI Group.
Limitations, reasons for caution: A larger prospective randomized multistudy would be required to confirm the findings of this study.
Wider implications of the findings: Our present findings together with previous observations suggest indirectly that sperm DNA integrity takes important role not only in fertilization moment but also in early embryo development.
Trial registration number: N/A.

O-192 Influence of sperm parameters on embryonic development, morphokinetics, and ART outcomes: male factor exploration in a female factor-controlled study platform

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Study question: Do sperm parameters influence embryonic development, morphokinetics, and ART outcomes in egg donation cycles?
Summary answer: High sperm apoptosis levels negatively influence good quality competent blastocyst production and ART outcomes in egg donation cycles.
What is known already: Infertility is a complex multifactorial phenotype where several elements, including male and female factors, could be involved. Accordingly, it is difficult to design controlled studies to anticipate which factors have a predominant effect on ART-outcomes. In order to investigate male factor impact on reproduction, an egg-donation program appears like the ultimate study platform. The number of studies of this kind is limited; to our knowledge none of them has focused on the effects of male parameters on embryo morphokinetics. The present study aims to shed some light into the influence of sperm quality on pre-implantation development morphokinetics and clinical outcomes.
Study design, size, duration: This unicentric and retrospective study included 282 sperm samples from patients undergoing egg-donation cycles and morphokinetic embryo selection using time-lapse imaging (Eeva™ test) at our centre between January 2014 and September 2015. The influence of sperm parameters in embryo development and ART outcomes is explored in a female factor-controlled study platform.
Participants/materials, setting, methods: Prior to fertilization, sperm concentration and motility based on WHO criteria (2010) and apoptosis levels using flow cytometry Annexin V-FITC/PI assay were determined in all samples.

All embryos were cultured until day 5 and monitored by time-lapse imaging (Eeva™) providing a prediction of embryo competency on day 3 (High/Medium/Low). Selected fresh single embryos were transferred to recipients in a hormonal substituted cycle. Reproductive outcomes were statistically compared between patients with normal and altered sperm values.
Main results and the role of chance: A significant impact on the production of good quality blastocysts with high implantation potential as predicted by Eeva test was observed in samples with altered levels of live apoptotic sperm cells (>30%). Samples with abnormal apoptotic levels showed a frequency of competent blastocysts significantly lower compared to samples with normal apoptosis values (26.54 ± 8.44 vs. 49.40 ± 2.57, $p = 0.028$). No impact of this parameter was observed on fertilization. Altered concentration or motility values were not correlated with either fertilization or embryo development rates.

In terms of clinical outcomes, the presence of apoptosis showed a dramatic negative effect on pregnancy rates. Biochemical pregnancy was significantly lower on samples with high levels of apoptosis (30.8 vs. 67.1%, $p = 0.009$). Similar results were found when clinical pregnancy rates (30.8 vs. 60.1%, $p = 0.04$) and ongoing pregnancy rates were compared (18.2 vs. 51.5%, $p = 0.036$). No such differences were detected when normal and altered sperm concentration or motility values were compared.

These results show that sperm apoptosis affects early stages of embryo development even when egg quality is good, and therefore can compromise the number of competent blastocysts obtained in egg-donation cycles. Moreover, sperm apoptosis levels negatively impact post-transfer embryo development even in cases were good quality embryos are transferred.
Limitations, reasons for caution: The retrospective and unicentric design of this study may be a reason of caution. The number of sperm samples with abnormal apoptosis levels was limited, this should be increased to confirm the results obtained. Further randomized studies are needed to validate our results.
Wider implications of the findings: Increased sperm apoptosis negatively influences blastocyst production and ART-outcomes in egg donation cycles. Reduction of apoptotic cell levels in the ejaculate by either medication or the use of MACS-selection technique may be a valuable strategy to improve embryo development and clinical outcomes in cases with high apoptotic sperm cell levels.
Trial registration number: A trial registration number was not required due to the retrospective study design.

SELECTED ORAL COMMUNICATIONS

SESSION 52: WHEN SPERM ARE THE LIMIT

Tuesday 05 July 2016 **Hall 5 CB** **17:00–18:00**

O-193 Limits for number of offspring in Danish anonymous sperm donors: what are the risks?

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Study question: How does the limit in the number of offspring from anonymous Danish sperm donors influence the risk and uncertainties of inbreeding?
Summary answer: We provide an improved statistical model that yields a recurrence time of 75 years for inbreeding with the current available Danish data.
What is known already: In 2013 the Danish limits for the number of offspring that a Danish sperm donor can father was lowered from 25 to 12. The choice for the new limit was not evidence-based, but was based on the feeling that the old limit was considered too high. The limit is set in place to reduce the risk of inbreeding and to minimize the risk that a sperm donor has an undiagnosed heritable disease that is segregated to a large number of offspring, but no-one has any good estimates of the actual risk in the Danish population.
Study design, size, duration: We used an improved version of the Hajnal–Curie–Cohen model to compute and evaluate the limits based on detailed information about the Danish population distribution drawn from Statistics Denmark.
Participants/materials, setting, methods: The Hajnal–Curie–Cohen (HCC) model is widely used to evaluate the average number of potential unwittingly consanguineous half-sibling matings among the offspring of an anonymous artificial insemination sperm donor. We extend the HCC model to accommodate detailed information about the donors and population, and improve it to make it possible to make statistical inference and evaluate different legal limits and requirements, and population restrictions.
Main results and the role of chance: We present the HCC model and our extensions and emphasize the three major improvements: (1) We can model the offspring of individual donors and not just the expected number of consanguineous

matings, which enables us to provide confidence and prediction intervals. (2) It accommodates differences in donor age distributions within and between the donor and natural offspring, and (3) the model allows us to directly evaluate the impact of legal requirements (e.g., limits based on offspring vs. families).

We find that the worst case scenario with the current limit of 12 offspring is substantially smaller ($p < 0.0001$) from the limits obtained from the improved HCC model (average recurrence time of 49 years for inbreeding in vs. 75 years), and that this difference is highly significant. In fact, our improved HCC model also provides a significant improvement in the risk estimation compared to the standard HCC model ($p < 0.01$).

We also find that a legal limit of 25 offspring per donor would still result in an average recurrence time of 21 years, which is hardly a substantial problem for the Danish society or gene pool.

Limitations, reasons for caution: This evaluation is based purely on a statistical model (albeit with detailed information from the population as input). Generally, the findings from these models is impossible to validate in the Danish (or any) population since the risk of unwittingly consanguineous half-sibling mating is extremely low.

Wider implications of the findings: The findings may influence the legal limits in Denmark, and the new statistical model may be used to make improved estimates, confidence intervals and prediction intervals of the risk of consanguineous mating among anonymous sperm donor offspring.

Trial registration number: None.

O-194 Microfluidic device based selective sorting of motile human sperm for IUI application: a preliminary study

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Study question: The purpose of the study is to develop a microfluidic chip for sorting highly motile and morphologically normal sperm for IUI applications.

Summary answer: The developed microfluidic device sorted sperm shows higher percentage of motile and morphologically normal sperm as compared to conventional methods, i.e., swim-up.

What is known already: According to the American Society for Reproductive Medicine, infertility affects about 5.3 million American couples of reproductive age, among which male infertility accounts for 40–50% of cases. The leading cause of male infertility is low sperm count, which is usually associated with low sperm motility, abnormality and impaired sperm function, thus resulting in the inability to fertilize an oocyte naturally. Assisted reproductive technologies (ARTs) have offered an alternative to address challenges associated with male infertility. A major clinical challenge is, however, the selection of highly motile and morphologically viable sperm to optimize the effectiveness of the procedures

Study design, size, duration: Sperm sorting analysis was performed using de-identified discarded human semen samples from REI Laboratory, Stanford School of Medicine, Stanford University. All the sperm processing experiments were performed with in 1–3 h after the semen samples were collected. The unprocessed semen sample was injected into microfluidic device to sort highly motile sperm. Similarly, unprocessed semen sample was also subjected to traditional swim-up method to compare the sperm sorting efficiency

Participants/materials, setting, methods: A microfluidic sperm-sorting device has two chambers (top and bottom) separated by a polycarbonate filter of various pore diameters, i.e., 8, 14, and 20 μm. Followed by the injection of semen sample into bottom chamber, top chamber was filled with human tubal fluid containing 4% human serum albumin, and device was incubated in 5% CO_2 at 37°C for 30 min. The motile and morphologically normal sperm were collected from top chamber for further analysis.

Main results and the role of chance: One unprocessed semen sample was collected and split two ways, i.e., swim-up and microfluidic device, which are analyzed using CASA system (CEROS by Hamilton Throne) to measure percentage of motile sperm. All the sperm functional parameters such as motility and morphology (based on Strict criteria defined by WHO) were analyzed and compared with sperm sorted using microfluidic device and swim-up approach. The results showed that the sperm sorted using 8, 14 and 20 μm pore membrane filtered microfluidic device have higher motility of 96, 90 and 92% respectively as compared to sperm sorted in swim-up approach (50%). Similarly, higher percentage of morphologically normal sperm was observed in sperm sorted using 8, 14 and 20 μm pore membrane filtered microfluidic device such as 58, 57 and 57% respectively, as compared to swim-up (34%). The higher motility and morphology in microfluidic device sorted sperm is due to the presence of micropores between the two chambers that selectively allow the most motile and morphologically viable sperm to swim offering a functional sorting mechanism over traditional sorting methods, i.e., swim-up.

Limitations, reasons for caution: The developed microfluidic sperm sorting device is optimized with 8 mm pore sized membrane, which shows better sperm sorting efficiency as compared to other pore sized membranes. On the other hand, sperm retrieval efficiency is increased with increasing the pore size of membrane that significant to improve clinical outcomes.

Wider implications of the findings: The developed microfluidic device is easy-to-use, high-throughput, and it can be an alternative approach for routine sperm sorting analysis to potentially improve fertilization outcomes.

Trial registration number: Not applicable.

O-195 Cryopreservation of very low numbers of spermatozoa from infertile men using agarose capsules

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Study question: Is it possible to cryopreserve low numbers of human spermatozoa from infertile men using agarose capsules?

Summary answer: We cryopreserved 2142 motile spermatozoa from 26 infertile male patients in 702 agarose capsules and 1356 (63%) were successfully recovered after thawing.

What is known already: Other methods for the cryopreservation of a few human spermatozoa have been reported. However, these methods are impractical due to difficulty in the preparation of materials or the time taken to search for spermatozoa after thawing. Therefore, we have devised a new method to cryopreserve a few spermatozoa using agarose capsules.

Study design, size, duration: Subjects were 26 infertile male patients at Tokuoka ladies clinic in 2015.

Participants/materials, setting, methods: We produced hollow core capsules with agarose walls. The capsules were transferred into a drop of cryoprotectant solution which contained 7% glycerol. We injected 3–4 motile spermatozoa, selected by swim up method, into agarose capsules using conventional ICSI procedure. These capsules were put on a Cryotop®, frozen in nitrogen vapor, and then submerged into liquid nitrogen and subsequently thawed and recovered. The motile spermatozoa in the capsules were counted.

Main results and the role of chance: The spermatozoa motility rates after thawing (MRAT) ranged from 20.0% (5/25) to 95.1% (58/61) among patients. The mean MRAT was 68.3%. Semen volume did not correlate with MRAT ($r = –0.075$). Spermatozoa concentration in the semen weakly correlated with MRAT ($r = 0.365$). Total number of motile spermatozoa in the semen moderately correlated with MRAT ($r = 0.493$). Total number of motile spermatozoa collected by swim up method strongly correlated with MRAT ($r = 0.746$).

Limitations, reasons for caution: None.

Wider implications of the findings: It was possible to cryopreserve infertile men's spermatozoa using agarose capsules. However, there were wide differences in MRAT among patients. It seems the oligozoospermic semen samples have a lower freezing resistance. Further studies using this method in cryptozoospermic semen samples, and spermatozoa taken from the testis or epididymis, are required.

Trial registration number: None.

O-196 Influence of the abstinence period on human sperm quality: analysis of 2,458 semen samples

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Study question: Is there a relationship between the sexual abstinence period and semen characteristics?
Summary answer: Increase in the sexual abstinence period influences sperm quality. Sperm DNA fragmentation and mitochondrial activity worsen, but chromatin packaging improves with an increased duration of abstinence.
What is known already: The duration of sexual abstinence that is needed to provide maximum sperm quality is one of the issues commonly discussed between physicians and patients. Most fertility clinics follow the World Health Organization (WHO) guidelines. However, there are few benchmark articles that have established current presumptions about the ideal duration of abstinence. The diversity regarding study designs used for assessing the influences of sexual abstinence on semen parameters and the size of the analysed population in the published reports are additional points that impair the conclusions.
Study design, size, duration: A cohort of 2,458 men undergoing infertility investigation was recruited. In the first evaluation, each man was asked to deliver a semen sample, and the abstinence period was recorded. No instruction about the abstinence period was given before semen collection for the study. Semen analyses were performed according to WHO guidelines/morphology-motile sperm organelle morphology examination/MSOME. For DNA integrity analysis, the percentages of DNA fragmentation (TUNEL), abnormal chromatin packaging/underprotamination (chromomycin A3/CMA3), abnormal mitochondrial membrane potential (MMP/MitoTracker Green), and apoptosis (annexin-V) were recorded.
Participants/materials, setting, methods: Associations between the sexual abstinence period and sperm parameters were assessed using Spearman correlation. For two-group comparisons, the subjects were categorized according to the sexual abstinence period (SAP) into three groups: SAP <2 days ($\mu = 0.8 \pm 0.3$), SAP 2–5 days ($\mu = 3.1 \pm 1.0$), and SAP >5 days ($\mu = 9.1 \pm 11.0$). At least 200 spermatozoa were examined in each evaluation. Potential confounders (age, BMI, smoking, alcohol, and varicocele) were also observed.
Main results and the role of chance: The duration of abstinence had a statistically significant positive influence on sperm concentration, volume, and the number of leukocytes and a statistically significant negative influence on sperm motility and vitality. The percentages of DNA fragmentation and MMP (mitochondrial damage) worsened with the increased duration of abstinence. The percentage of sperm protamination was statistically significantly increased with abstinence (Tables 1 and 2).

No correlation between abstinence and potential confounders was observed.

Table 1. Sexual abstinence × semen parameters: Spearman's correlation.

Outcomes	Correlation coefficient	*p*
pH	−0.01	0.52
Volume (ml)	0.10	0.0004
Concentration (ml × 10⁶)	0.12	<0.0001
Progressive motility (%)	−0.10	0.0007
Normal forms (%)	0.03	0.12
Leukocytes (×10⁶/ml)	0.10	0.0001
Vitality (%)	0.12	<0.0001
DNA fragmentation (%)	0.12	<0.0001
Apoptosis (%)	0.01	0.21
CMA3 positivity (%)	−0.12	<0.0001
Abnormal MMP (%)	0.10	0.003

Table 2. Sexual abstinence × semen parameters: group comparisons.

Outcomes	SAP: <2 days	SAP: 2–5 days	SAP >5 days	*p*
n	244	1.932	282	
pH	8.0 ± 0.6	8.0 ± 0.5	8.0 ± 0.4	0.28
Volume (ml)	2.3 ± 1.2[a,b]	2.7 ± 1.6[a]	2.8 ± 1.7[b]	0.0001[a], 0.0004[b]
Concentration (ml × 10⁶)	60.7 ± 50.0[a,b]	70.4 ± 61.1[a,c]	92.1 ± 65.8[b,c]	0.02[a], <0.0001[b,c]
Progressive motility (%)	58.3 ± 16.2[a,b]	56.1 ± 16.9[a,c]	53.8 ± 16.1[b,c]	0.03[a], 0.0004[b], 0.01[c]
Normal forms (%)	0.7 ± 1.0	0.9 ± 1.4	1.0 ± 1.8	0.77
Leukocytes (×10⁶/ml)	0.3 ± 0.6[a,b]	0.4 ± 0.9[a]	0.5 ± 1.2[b]	0.002[a], 0.0002[b]
Vitality (%)	66.8 ± 14.0[a,b]	65.0 ± 15.0[a,c]	61.3 ± 14.8[b,c]	0.03[a], <0.0001[b,c]
DNA fragmentation (%)	14.5 ± 8.2[a]	15.3 ± 8.4[b]	17.1 ± 9.0[a,b]	0.001[a], 0.002[b]
Apoptosis (%)	19.3 ± 8.5	19.1 ± 7.9	20.2 ± 7.3	0.17
CMA3 positivity (%)	59.8 ± 15.3[a,b]	56.1 ± 15.2[a,c]	53.2 ± 14.2[b,c]	0.005[a], 0.0002[b], 0.02[c]
Abnormal MMP (%)	23.3 ± 14.0[a]	25.6 ± 16.5	28.6 ± 17.5[a]	0.01[a]

Limitations, reasons for caution: The study involved samples from men attending a fertility clinic who are likely to have a lower semen quality and higher rate of pathology compared with the general population.
Wider implications of the findings: This study reinforces the importance of the duration of ejaculatory abstinence on semen parameter variation. It highlights the deleterious effect of increased abstinence on DNA damage, which is most likely associated with ROS (mitochondrial damage/number of leukocytes). The increase in chromatin packaging can represent a protective feature for DNA.
Trial registration number: Not applicable. The local ethics committee authorised this study.

SELECTED ORAL COMMUNICATIONS

SESSION 53: GENETIC FACTORS IN INFERTILITY

Tuesday 05 July 2016 **Hall 5 A** **17:00–18:00**

O-197 Identifying Clock genes and comparing their circadian expression in the endometrium of healthy fertile women and those with recurrent implantation failure (RIF)

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Study question: Are Clock genes expressed in a rhythmic manner in the endometrium of fertile women and those with RIF, and does decidualisation of the cells have an impact on this expression?
Summary answer: Clock genes are expressed in decidualised and non-decidualised endometrium. This rhythmicity may be influenced by pathology within the endometrium of women with reproductive problems.
What is known already: Many aspects of reproductive function are strongly circadian but the rhythmicity that happens within the reproductive organs themselves is less well understood. The majority of evidence from mammals suggests that female reproductive structures contain circadian clocks and Clock genes are expressed throughout the hypothalamic–pituitary–gonadal (HPG) axis.

Endometrial dysfunction may occur if regulatory endocrine stimulation fails, either centrally or peripherally, with a disruptive effect on the synchronisation of implantation. Whether this dysregulation happens at the level of the endometrium, which may be altered by the relative expression and timing of clock genes, or at the embryo-endometrial interface is not known.
Study design, size, duration: A case–control gene expression study (August 2013–2015) was performed *in vitro* to quantify the levels of Clock gene expression in human endometrial tissue. The level of expression was measured every 4 h over a 36 h period (in decidualised and non-decidualised endometrial stromal cells, total of 80 samples) to investigate how expression altered over time. The affect of adding progesterone was compared with untreated cells in healthy endometrial tissue and in those women with RIF.
Participants/materials, setting, methods: Women between 25 and 45 years were recruited and baseline demographics and fertility characteristics were collected. Women were included if they suffered RIF or were fertile controls. Endometrial biopsies were taken by suction curette, the stromal cells were isolated and cultured *in vitro* before decidualisation was commenced by the addition of

progesterone. Cells were sampled at baseline and four-hourly intervals. Total RNA extraction, cDNA synthesis and PCR of Clock-controlled genes was performed.

Main results and the role of chance: Six Clock-controlled genes (CLOCK, BMAL, PER1, PER2, CRY1, CRY2) are present and are cyclically expressed in endometrial tissues *in vivo.* This expression level varies over the period of a day. Decidualisation appears to alter the relative expression of these genes and this effect continues over time (up to 36 h) without resynchronising the peripheral "Clock" and independently of central control.

The endometrium from fertile women displays a different rhythmicity of these genes compared with those women who have suffered RIF. This suggests that any underlying pathology in RIF women may be related to dysynchrony at the level of the gene regulation within the endometrium. Whether or not this effect remains over time periods longer than 36 h is yet to be elicited.

The timing of events at the embryo-endometrial interface during the reproductive cycle is not fully understood and the expression of the Clock-controlled genes may have a role to play. Future work should be aimed at demonstrating the level of this control and whether it may be manipulated to beneficial effect.

Limitations, reasons for caution: Our results may be confounded by a small sample size; a larger scale study in this specific subgroup of women is further needed to confirm our findings.

Wider implications of the findings: Clock-controlled genes are found within the endometrium and are affected by the effects of progesterone. The manner in which these genes are expressed is also related to reproductive pathology. Cellular functions of clock genes may have a role in RIF and the process of embryo implantation.

Trial registration number: Regional ethics committee number 12/SC/0568.

O-198 Hyperandrogenism reprograms leptin *via* down-regulation of DNMT1, a novel mechanism involved in decreased pregnancy rates in PCOS patients

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Study question: How does hypernadrogenism affect leptin reprogramming in human granulosa cells, subsequently alter leptin levels and impact female reproductive health?

Summary answer: Dihydrotestosterone increased Leptin expression and decreased methylation level in *leptin* promoter in both *in vivo* and *in vitro* experiment, which is associated with failed pregnancy.

What is known already: Hypernadrogenism is the main pathologic characteristic of polycystic ovary syndrome (PCOS). Hyperleptinemia and high follicular fluid (FF) leptin levels are important pathologies in PCOS patients with infertility.

Study design, size, duration: A case control study was performed among 43 women with hyperandrogenism and 159 infertile women with tubal factor (control) from January to June 2015.

Participants/materials, setting, methods: The diagnosis of PCOS was made according to the Rotterdam Consensus. The subjects were divided into successful and failed subgroups according to *in vitro* fertilization outcomes. We examined testosterone and leptin levels in serum and follicular fluid using ELISA, real-time PCR. Bisulfite Sequencing PCR were performed to detect Leptin expression and methylation level in granulosa cells, also effect of dihydrotestosterone *in vitro*. Expression of DNA methyltransferase 1 was also detected *in vivo* and *in vitro*.

Main results and the role of chance: Leptin levels in serum and follicular fluid of women with hyperandrogenism were significantly higher than control, serum leptin level was positively correlated with testosterone level. *In vitro* study showed that Dihydrotestosterone (DHT) increased Leptin expression and decreased methylation level in Leptin DMR in primary granulosa cells (GCs), whereas DHT decreased the expression of DNA methyltransferase 1 (DNMT1) both *in vivo* and *in vitro*. Further analysis revealed that women from successful pregnancy group showed significantly lower Leptin and testosterone levels. Hyperandrogenism rat also showed increased Leptin expression accompanied with decreased methylation level in Leptin DMR and decreased DNMT1 expression in GCs.

Limitations, reasons for caution: Hypernadrogenism and high leptin levels were coexisted in follicular fluid. The relationship between them hasn't been proven in this study. Further study is needed.

Wider implications of the findings: Hyperandrogenism increased leptin expression *via* hypomethylation of *leptin* promoter, probably attributed to decreased DNMT1. Up-regulation of leptin was associated with failed pregnancy in patients with hyperandrogenism.

Trial registration number: ChicCTR-OCH-14004537, www.medresman.org.

O-199 Differentially methylated LINE1 patterns in sperm DNA of infertile men

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Study question: To investigate the overall methylation and the patterns of methylation of two consecutive CpGs within the LINE-1 retroelements in sperm from normospermic and oligospermic men.

Summary answer: Our data show significant differences in the overall methylation levels and in the DNA methylation patterns from spermatozoa of normospermic and oligospermic men.

What is known already: DNA methylation in mammals is a dynamic and strictly controlled process, in numerous cellular processes, cell differentiation, regulation of gene expression, genome reprogramming and silencing of repetitive elements. In mammals, epigenetic reprogramming is crucial for germ cell development. Principal epigenetic regulatory mechanism is DNA methylation during gametogenesis and early embryo preimplantation stages. Differentially methylated regions in sperm DNA involve mainly the retroelement LINE-1, which is abundant in the human genome, with 5×10^4 copies. LINE-1 is silenced during gametogenesis by DNA methylation, chromatin modifications and Piwi-interacting RNAs. Aberrant DNA methylation levels of LINE-1 have been implicated in various human disorders.

Study design, size, duration: The study included 92 men who gave a detailed medical history. Subjects were stratified in two groups: (a) normospermic (70 men) and (b) oligospermic (22 men, $<15 \times 10^6$ spermatozoa). The oligospermic group was further classified into moderate (13 men, $5–15 \times 10^6$ spermatozoa) and severe (9 men $<5 \times 10^6$ spermatozoa). LINE-1 overall methylation levels and methylation patterns of the oligospermic group were compared to the normospermic control group.

Participants/materials, setting, methods: DNA methylation levels and patterns of LINE-1 were measured by the semi-quantitative COBRA method. DNA extracted from human sperm, was treated with sodium bisulfate and amplified using primers designed to amplify the 5′UTR. Amplicons were digested with methylation sensitive enzymes, and electrophoresed in polyacrylamide-gels. Intensities of the bands were measured by a phosphoimager. COBRA assays were performed in duplicates with DNA from Hela, Jurkat and Daudi cells as controls.

Main results and the role of chance: Semi-quantitative COBRALINE-1 results were sorted as four distinct methylation patterns of the two CpG dinucleotides in the 5′UTR: hypermethylation ($^{m}C^{m}C$), hypomethylation ($^{u}C^{u}C$), partial methylation of the 5′($^{m}C^{u}C$) and partial methylation of the 3′end ($^{u}C^{m}C$).

Significant differences ($p = 0.039$) in the overall methylation levels of LINE-1 in the normospermic and severe oligospermic samples were found. In the severe oligospermic samples the overall methylation level (41.52%) was significantly higher than in the normospermic samples (40.62%).

Pattern analysis in normospermic samples found statistically significant correlation of the partially methylated $^{m}C^{u}C$ and the hypomethylated $^{u}C^{u}C$ motif ($p = 0.031$). Although we found a direct correlation ($r = 0.258$) between the two patterns in normospermics there was no such significant result in oligospermics.

Analysis in oligospermic samples found statistically significant inverse correlation ($r = -0.526$) between the partially methylated pattern $^{m}C^{u}C$ and the hypermethylated $^{m}C^{m}C$ ($p = 0.012$) in contrast to normospermics.

In addition, the partially methylated pattern $^{u}C^{m}C$ and the hypermethylated $^{m}C^{m}C$ were inversely correlated in both oligospermics and normospermics ($p = 0.003$, $r = -0.602$ and $p = 0.0001$, $r = -0.625$ respectively).

Limitations, reasons for caution: A major limitation is the small number of oligospermic subjects. The semi-quantitative COBRA method is also a limitation and a fully quantitative method would provide more accurate results.

Wider implications of the findings: Our findings indicate that normospermics have lower methylation levels and different methylation patterns of LINE-1 in comparison to oligospermics. Differences may reflect abnormalities in the initial demethylation process at Primordial Germ Cells rather than abnormalities in the reestablishment of methylation during sperm maturation.

Trial registration number: –.

O-200 Methylation of a novel FMR1 epigenetic element depends on women ovarian reserve

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Study question: We want to study whether FMR1 (Fragile × Mental Retardation 1) epigenetic control in human granulosa cells (GC) have an impact on women ovarian response.

Summary answer: The identification of a novel FMR1 epigenetic element (FREE3) within the promoter whose methylation pattern varies according to ovarian response

What is known already: The FMR1 gene contains in exon1 a repetitive CGG-triplet block ($n = 26$–34 in general population) which is a part of its promoter CpG island. The implication of FMR1 in human folliculogenesis is highlighted by the fact that women with increased CGG triplet numbers have increased risk of primary ovarian insufficiency/failure (POF/POI) (when: $52 \leq n \leq 200$ increase to 20%). Recent molecular studies showed that FMR1 CpG island is also under complex epigenetic control; two epigenetics elements called FREE1 and FREE2 (Fragile × epigenetic element 1 and 2) were indicated as predictors of FMR protein (FMRP) expression level in female leukocytes (CGG >52).

Study design, size, duration: Establishment of a specific CpG Methyl Specific Primers (MSP) assay to reveal allele specific CpG methylation pattern within the 5′FMR1 CpG island promoter region including its first intron in patients primary GC.

Patients seeking *In Vitro* Fertilization (IVF) treatment were divided into three groups according to their ovarian response; High (HOR), Normal (NOR) and Poor (POR) Responders (according to Bologna Criteria). CpG methylation profiles were compared between groups for statistically significance using the Mann–Whitney-*U* test.

Participants/materials, setting, methods: According to ovarian response after controlled ovarian stimulation, granulosa cells from women with different ovarian response were collected by our IVF laboratory and used for MSP assays. Human GC line COV434 was used in parallel as model system investigating the FMR1 promoter and first intron CpG methylation profile.

Main results and the role of chance: The data point to a similar complex epigenetic control in GCs as reported earlier in leukocytes. FREE1 and FREE2 were found in the COV434 cell line as well as in primary GCs from patients. Additionally, a third epigenetic element was identified in intron 1 called accordingly FREE3. Using the Mann–Whitney-*U* test significant divergent FREE3 CpG methylation patterns for specific CGs was found between HOR/NOR/POR patients groups, i.e., this pattern was dependent on ovarian response. It suggests that this CG specific methylation profile have an impact on FMRP expression level in GCs.

Limitations, reasons for caution: Our preliminary data were obtained from FREE3 sequencing results of 25 patients. Screening a higher number of patients with different ovarian reserve is going on by using a high throughput CpG methylation analysis to confirm the obtained data.

Wider implications of the findings: For the three patient groups FREE3 shows a similar divergent CG methylation profile in both leukocytes and granulosa cells. If this holds true our MSP assay for FREE3 can be used as a non-invasive prognostic tool in leukocytes to predict ovarian reserve and response in females entering the infertility clinic.

Trial registration number: This study was funded by the Deutsche Forschungsgemeinschaft (DFG) to J. Rehnitz.

SELECTED ORAL COMMUNICATIONS

SESSION 54: REPRODUCTIVE SURGERY

Tuesday 05 July 2016 **Hall 3 AB** **17:00–18:00**

O-201 Hysteroscopic intratubal Essure® device placement versus laparoscopic salpingectomy, as treatment for hydrosalpinges prior to *in vitro* fertilization (IVF)/intracytoplasmatic sperm injection (ICSI): a randomised controlled trial

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[5]*The Robinson Research Institute, School of Paediatrics and Reproductive Health, Adelaide, SA, Australia*

Study question: Does hysteroscopic proximal tubal occlusion by Essure® devices as treatment for hydrosalpinges result in comparable ongoing pregnancy rates following IVF/ICSI as compared to laparoscopic salpingectomy?

Summary answer: Hysteroscopic proximal tubal occlusion by Essures® is inferior to laparoscopic salpingectomy in the treatment of hydrosalpinges in women undergoing IVF/ICSI with respect to ongoing pregnancy.

What is known already: It is known that women with hydrosalpinges undergoing IVF have poorer pregnancy outcomes compared to women with other forms of tubal infertility. In these women both laparoscopic salpingectomy and laparoscopic proximal tubal ligation are known to improve IVF outcomes. At present it is unclear whether a less invasive hysteroscopic treatment with Essure® leads to similar ongoing pregnancy rates following IVF as compared to laparoscopic salpingectomy which is currently considered as standard treatment.

Study design, size, duration: A two-centre, randomised controlled non-inferiority trial. Between October 2009 and December 2014 a total of 85 women were included in this study, of whom 42 were randomised to hysterocopic Essure® treatment and 43 to laparoscopic salpingectomy. Randomization was based on a computer generated randomization list. The study was unblinded. Primary outcome was ongoing pregnancy rate, defined as a fetal heartbeat on ultrasound beyond 10 weeks gestation following one IVF/ICSI treatment (fresh and frozen-thawed embryo transfers).

Participants/materials, setting, methods: We studied women between 18 and 41 years, with uni- or bilateral ultrasound visible hydrosalpinges who were scheduled for an IVF/ICSI treatment.

Main results and the role of chance: The ongoing pregnancy rates according to the intention to treat principle were 11/42 (26.2%) after Essure® (intervention group) versus 24/43 (55.8%) after laparoscopic salpingectomy (control group) (absolute difference 29.6%; 95% CI 7.1–49.1, RR 0.47 95% CI 0.27–0.83). In the per protocol analysis the ongoing pregnancy rate following Essure® treatment was 9/27 (33.3%) compared to 19/32 (59.4%) following laparoscopic salpingectomy (absolute difference 36.1%; 95% CI −1.8 to 50.0, RR 0.56; 95% CI 0.31–1.03).

Limitations, reasons for caution: Masking participants and investigators would be difficult due to the nature of both interventions and since we had objective outcome measurements we witholded sham procedures, leaving the study unblinded. Furthermore our low sample-size resulted in wide confidence intervals. A larger sample size would result in a more accurate treatment effect.

Wider implications of the findings: In the treatment of hydrosalpinges prior to IVF/ICSI, hysteroscopic proximal tubal occlusion by Essure® is

inferior to laparoscopic salpingectomy. Therefore, Essure® treatment should only be considered in women with hydrosalpinges who are laparoscopically inaccessible.

Trial registration number: The Dutch Trial Register: NTR 2073.

O-202 Laparoscopy vs. robotic surgery for endometriosis (LAROSE): a multicenter randomized controlled trial

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[2]*Cleveland Clinic, Obstetrics, Gynecology and Women's Health Institute, Cleveland, OH, USA*
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[4]*Mayo Clinic Arizona, Gynecology, Scottsdale, AZ, USA*
[5]*Harvard/Brigham and Women's Hospital, Minimally Invasive Gynecology, Boston, MA, USA*

Study question: Is robotic surgery comparable to laparoscopy for the treatment of endometriosis?

Summary answer: There were no clinically significant differences between the laparoscopy and robotic surgery groups with regards to operative time, postoperative pain or quality of life.

What is known already: Retrospective studies in the literature suggest that robotic surgery is comparable to laparoscopy for the treatment of endometriosis but the former is associated with greater operative time. One study revealed a shorter robotics operative time for patients with advanced endometriosis (stage III and IV).

Study design, size, duration: A prospective, multicenter, randomized blinded controlled trial was performed in 73 patients between March 2012 and December 2015. Patients from Cleveland Clinic, Harvard/Brigham and Women's Hospital and Mayo Clinic Arizona were randomized at the time of surgery to laparoscopy (LSC) vs. robotic surgery (ROB) to treat presumed endometriosis. The primary outcome was operative time. Power calculation revealed that 37 patients were needed in each arm of the study ($\beta = 0.8$, $\alpha < 0.05$).

Participants/materials, setting, methods: Patients ≥18 years old that were to have minimally-invasive surgery for presumed endometriosis were randomized to laparoscopy or robotic surgery ($n = 73$). Perioperative variables recorded included operative time, estimated blood loss, perioperative complications, hospital stay and pain medication use.

Validated quality of life questionnaires -SF-12 and Endometriosis Health Profile-30 (EHP-30)-, pain medication use questionnaire and pain and activity scales were applied preoperatively and at 6 weeks and 6 months after surgery.

Main results and the role of chance: A total of 73 patients were included in the study (LSC: 35, ROB: 38). One patient from the robotic arm withdrew from the study. Mean age, body mass index, indication for surgery and other baseline characteristics were comparable in both groups. The mean operative time (SD) was similar in the laparoscopy and robotic surgery groups [101.6 (63.2) min, 106.6 (48.4) min, $P = 0.71$]. The estimated blood loss was comparable in both groups (43.8, 100.9 cc, $P = 0.136$). Intraoperative complications, length of hospital stay, postoperative complications and use of postoperative pain medication was comparable in both groups. Preoperative pain scores were similar in both groups (LSC: 51.2 ± 18.1 vs. ROB: 52.8 ± 17, $P = 0.44$). There was a progressive decrease in pain scores observed at 6 weeks and 6 months in both arms without statistically significant differences between groups [6 weeks, LSC: 22.7 (6.8, 40.9) vs. ROB: 25.0 (4.5, 43.2), $P = 0.79$; 6 months, LSC: 11.4 (0.0, 45.5) vs. ROB: 13.6 (0.0, 50.0), $P = 0.43$]. Patients in the laparoscopy arm had a higher SF-12 Physical Health Scores in comparison to patients in the robotic arm at 6 weeks [41.9 (2.8) min, 39.6 (3.6) min, $P = 0.01$]; SF-12 Physical and Mental Scores and other quality of life scores (EHP-30) were comparable in both groups at 6 months.

Limitations, reasons for caution: A limitation of the study is that the sample size may not be large enough to detect differences in the secondary outcomes. A patient from the robotic arm withdrew from the study after randomization.

Wider implications of the findings: The observations of the current study suggest that similar outcomes can be achieved with robotic or laparoscopy for the treatment of endometriosis when the surgery is performed by surgeons that are proficient in both approaches. Traditional laparoscopy should still be considered the gold standard approach for endometriosis.

Trial registration number: ClinicalTrials.gov identifier: NCT01556204.

O-203 Hysteroscopic removal of placental remnants: a randomised controlled trial comparing hysteroscopic morcellation with cold loop resection

T. Hamerlynck[1], H. van Vliet[2], A.S. Beerens[3], S. Weyers[1], B. Schoot[2]
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[3]*Ghent University Hospital, Department of Pathology, Gent, Belgium*

Study question: Is hysteroscopic morcellation a better technique for hysteroscopic removal of placental remnants compared with cold loop resection?

Summary answer: Hysteroscopic morcellation is faster than cold loop resection, and both techniques show high rates of complete removal and a low risk of intrauterine adhesion formation.

What is known already: Removal of placental remnants by curettage is common practice, but it is associated with postoperative intrauterine adhesions in up to 40%. Alternatively, cold loop resection and hysteroscopic morcellation allow for mechanical, selective removal of placental remnants under direct vision, and hysteroscopic removal appears favourable to curettage in cohort studies, with more complete evacuations, less intrauterine adhesions, and similar reproductive outcomes.

Study design, size, duration: From May 2011 to July 2015 an open, randomised controlled trial was performed comparing hysteroscopic morcellation with the TRUCLEAR 8.0 Tissue Removal System with cold loop resection using a rigid 8.5 mm bipolar resectoscope for removal of placental remnants. The sample size was calculated at 34 women in each group. Randomisation was done by a computer generated random allocation sequence. Eighty-six women with placental remnants after pregnancy were included in the intention-to-treat analysis.

Participants/materials, setting, methods: Patients with placental remnants diagnosed by ultrasound and/or diagnostic hysteroscopy were randomly allocated to either removal by hysteroscopic morcellation or cold loop resection. Patients were treated in day surgery under spinal or general anaesthesia. Routine second-look ambulant hysteroscopy was performed to study postoperative adhesion formation. Both techniques were compared in terms of procedure time, adverse events, tissue availability, histology results, short-term effectiveness and postoperative adhesion formation.

Main results and the role of chance: Median operating time was significantly reduced in the hysteroscopic morcellation group compared with the cold loop resection group [6.2 min (4.0–11.2 min) vs. 10.0 min (5.8–16.4 min), $p = 0.023$]. After correction for diameter, both operating time and total procedure time were significantly less for hysteroscopic morcellation compared with cold loop resection, with a reduction of 40% (95% CI 15–58%, $p = 0.005$) and 22% (95% CI 5–37%, $p = 0.014$), respectively. No technique related adverse events occurred. However, 8 cases of perforation at dilation were seen in the hysteroscopic morcellation group, resulting in 2 procedure discontinuations and 1 incomplete removal in that group. Incomplete removal was found in, 1 uncomplicated procedure of the hysteroscopic morcellation group and 2 uncomplicated procedures of the resection group. Tissue was available for pathology analysis in 40/44 (91%) and 37/39 (95%) of cases with placental remnants present at the time of the operation in the hysteroscopic morcellation and resection group, respectively, showing positive histology in 27/40 (67.5%) and 26/37 (70%). De novo intrauterine adhesions were seen in 1/35 patients (3%) of the hysteroscopic morcellation group, and 1/30 (3%) patients of the resection group, during second-look hysteroscopy.

Limitations, reasons for caution: The study was powered for procedure time only. Patients and surgeons were not blinded for the procedure. Although the majority of patients underwent second-look ambulant hysteroscopy after removal of placental remnants, 18% of patients did not. This may have led to selection bias considering the outcome of intrauterine adhesions.

Wider implications of the findings: Hysteroscopic morcellation is a faster alternative than cold loop resection, and both techniques are effective and safe in removing placental remnants. Prospective randomised comparison between hysteroscopic treatment and curettage is needed to confirm the expected advantages of removal of placental remnants under direct vision in women with future reproductive desire.
Trial registration number: NCT01537822.

O-204 Natural pregnancy rates after fallopian tube catheterisation for proximal tubal obstruction: a systematic review and meta-analysis

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Study question: What is the chance of natural clinical pregnancy when fallopian tube catheterisation (FTC) is used for unilateral or bilateral proximal tubal occlusion (PTO)?
Summary answer: The pooled natural clinical pregnancy rate (CPR) of FTC for PTO is 27% (95% CI 25–30%).
What is known already: Tubal disease accounts for approximately 30% of female infertility and is caused by infection, endometriosis or pelvic surgery. FTC, using a guidewire to direct a fine catheter into the fallopian tube, can be carried out under laparoscopic, hysteroscopic, fluoroscopic or ultrasonic guidance. This technique has fallen out of favour since the advent of *in vitro* fertilisation (IVF). Whilst many cohort studies have investigated the success of FTC, there have been no randomised studies comparing FTC with IVF to ascertain the optimal management of women with PTO. Our meta-analysis is the first to investigate reproductive outcome following FTC for PTO.
Study design, size, duration: Our literature search identified 26 cohort studies from 1989-2015, comprising 1530 patients who underwent FTC for PTO, all attempting natural conception. In the absence of any randomised controlled trials comparing FTC with IVF for PTO, we meta-analysed all suitable observational studies that met our inclusion criteria.
Participants/materials, setting, methods: Systematic literature searches using MEDLINE, EMBASE and Cochrane Central Register of Controlled Trials, were performed, returning a total of 2190 studies. Only studies that reported FTC outcome alone were included. Outcomes after FTC from PTO due to iatrogenic causes such as microsurgery or sterilisation were excluded. 26 cohort studies matched the strict inclusion criteria for data extraction in the meta-analysis.
Main results and the role of chance: The meta-analysis showed a pooled natural CPR of 27% (95% CI 25–30%) after the use of FTC for unilateral or bilateral PTO (26 studies, 1530 patients). In women with bilateral PTO (14 studies, 617 patients), the CPR was 27% (95% CI 23–32%).

In women undergoing FTC, meta-analysis of natural CPRs demonstrated that the cumulative CPRs were 22.3% (95% CI 17.8–27.8%) at 6 months, 25.8% (95% CI 21.1–31.5% CI) at 9 months, 26.4% (95% CI 23.0–30.2%) at 12 months, 25.8% (95% CI 22.6–29.6%) at 18 months, 26.9% (95% CI 23.9–30.4%) at 24 months, 27.8% (95% CI 24.8–31.3%) at 36 months and 28.4% (95% CI 25.4–31.7%) at 48 months.

Furthermore, the pooled live birth rate (13 studies, 525 patients) was 22% (95% CI 18–26%). The pooled miscarriage rate (15 studies, 633 patients) was 6% (95% CI 4–8%). The pooled ectopic pregnancy rate (26 studies, 1530 patients) was 4% (95% CI 3–5%).

The included studies scored satisfactorily on the Newcastle–Ottawa quality assessment scale.
Limitations, reasons for caution: The pooled natural CPR after FTC was found to be almost comparable to that after IVF (approximately 35%). However, included studies were small, non-comparative series with significant clinical heterogeneity in population characteristics, surgical technique and follow up.
Wider implications of the findings: In light of great disparity in the number of IVF cycles the National Health Service provides in the UK, these findings suggest FTC as an alternative strategy or adjunct in patients presenting with PTO. Further research is needed to identify particular patient sub-groups that would benefit the greatest from FTC.
Trial registration number: N/A.

SELECTED ORAL COMMUNICATIONS

SESSION 55: FEMALE FERTILITY. NEW AND RENEWED IDEAS

Tuesday 05 July 2016 **Hall 3 DE** **17:00–18:00**

O-205 Impact of serum vitamin D levels on ovarian reserve and ovarian response to ovarian stimulation in egg donors

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Study question: Is there any correlation between serum vitamin D levels and the ovarian response to ovarian stimulation in healthy oocyte donors?
Summary answer: Serum vitamin D levels from oocyte donors was unrelated to their ovarian reserve or reproductive outcomes in oocyte recipients.
What is known already: Vitamin D deficiency is very common all over the world.

Some studies suggested a correlation between vit D deficiency and lower fertility; however infertility is diagnosed in around 15% of the couples, so the causative role of the vitamin D deficiency is still unclear.

A few publications demonstrated a reduction in the implantation and pregnancy rates in patients with non-replete vitamin D status after *In Vitro* Fertilization or egg donation, while others found no impact.

Only two groups have described a positive correlation between serum vitamin D and AMH levels while another group found an inverse correlation with serum FSH.
Study design, size, duration: We designed a retrospective study to investigate the association between serum vitamin D levels and ovarian reserve in oocyte donors and the reproductive outcome in the corresponding recipients.

In this study we included 994 healthy oocyte donors from June 2014 to April 2015.
Participants/materials, setting, methods: We selected 994 healthy donors between 18 and 34 years and we stimulated them using recFSH under GnRH antagonist and agonist triggered in our center.

Vitamin D binding protein measurement was performed by a commercial ELISA kit.

Vitamin D was determined by chemiluminescence by using a Advia Centaur analyzer.

Albumin determination was realized by using a Cobas Mira Plus analyzer.

Statistical analysis was performed by using SPSS program. Student's *t* test and ROCC curves were used as appropriate.
Main results and the role of chance: Among all donors, 288 were vitamin D replete (vitamin D >30 ng/ml), 524 had vitamin D insufficiency, and 182 presented deficiency (<20 ng/ml).

Days of stimulation, estradiol levels on hCG day, number of follicles, oocytes retrieved, number and rate of mature oocytes and cycle characteristics were comparable in the three groups.

Oocyte recipients that received embryos from vit D deficient donors obtained a lower number of embryos when compared to the ones who received embryos from a vitamin D replete donors ($p < 0.001$).

However, we did not find differences between the three groups of receptors in terms of implantation ($p = 0.48$), pregnancy ($p = 0.11$), ongoing pregnancy ($p = 0.6$) and miscarriage rate ($p = 0.6$).
Limitations, reasons for caution: Although this is a very large series, the retrospective nature of our study requires prospective validation.
Wider implications of the findings: Patients who receive oocytes from vitamin D deficient donors don't have a lower chance of becoming pregnant.

This highly prevalent vitamin D insufficiency does not impair ovarian reserve and/or quality in egg-donors.

Actually the recommendation of analyzing vitamin D status screening in egg-donors is not supported by our evidence
Trial registration number: 1508-MAD-055-JG.

O-206 The impact of acupuncture on IVF success rates: a randomized controlled trial

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Study question: To examine in a sufficiently powered prospective randomized controlled trial, whether acupuncture during IVF treatment can improve IVF success rates.
Summary answer: Acupuncture during IVF treatment significantly improves IVF success rates compared with a basically similar control group having no acupuncture.
What is known already: Clinical trials to assess the benefits of acupuncture on IVF treatment results have differed in study design, protocol, outcome measures and commercial bias. This heterogeneity has precluded any firm conclusion regarding the efficacy or otherwise of acupuncture in this field. To address this heterogeneity, 15 international acupuncturists participated in Delphi questionnaires and reached a consensus protocol to be used in future research which, so far, has not been implemented. To the best of our knowledge, this is the first study to use this newly agreed standard protocol.
Study design, size, duration: In this single-centre RCT, conducted over 1 year, according to the power calculation 160 couples were randomized into two groups using computer generated randomization numbers distributed consecutively by central telephone. One group received acupuncture treatment 4 times spread throughout the treatment cycle in addition to our standard IVF protocol. The other group went through the identical standard protocol but with no acupuncture.
Participants/materials, setting, methods: All couples in the study were undergoing their 1st or 2nd IVF cycle, age 23–43, BMI < 30 and consented to randomization before their treatment cycle. Those randomised to the study group ($n = 80$) received acupuncture based on the Delphi consensus protocol, between days 6 and 8 of ovarian stimulation, before egg retrieval and twice, before and after, embryo transfer. For both groups the IVF protocol was decided by the clinician, blinded to randomization. Primary end point was live birth.
Main results and the role of chance: To date, a total of 127 results are available and have been analysed, 67 in the acupuncture (study) group and 60 controls. So far, the total ongoing pregnancy rate in the two groups is 34.6%. This comprises an ongoing pregnancy rate of 31/67 (46.2%) in the acupuncture group and 13/60 (21.7%) in the controls ($P = 0.004$). Two couples withdrew from the control group. Live birth rates are being closely followed up and will be reported.
Limitations, reasons for caution: The additional attention paid to the acupuncture group as opposed to controls may have had a positive psychological influence.
Wider implications of the findings: The results of this study imply that acupuncture may be offered as a possible method of improving IVF outcome. This study is the first to follow a widely approved consensus protocol and therefore has settled disagreement in the literature and resolved previous disparity.
Trial registration number: 13/LO/1356.

O-207 Effectiveness of different combined oral contraceptives – final results from the "international active surveillance study – safety of contraceptives: role of estrogens" (INAS-SCORE) Study

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Study question: The secondary outcome focuses on unintended pregnancies and is compared between the two user cohorts: DNG/EV and established COCs in the US and Europe.
Summary answer: Combined oral contraceptives (COCs) have a high contraceptive effectiveness, nevertheless, lower contraceptive failure rates were seen in the European DNG/EV cohort compared to other COCs.
What is known already: Oral contraceptives are the most popular method of birth control and widely used in the USA and Europe. DNG/EV (Qlaira/Natazia) is a new combined oral contraceptive (COC) that contains estradiol valerate and dienogest. Up to date, there are no comparative data on the contraceptive effectiveness of DNG/EV and other COCs.
Study design, size, duration: INAS-SCORE was a large, prospective, controlled, non-interventional, long-term cohort study carried out in the US as well as in Austria, France, Germany, Italy, UK, Poland and Sweden. It was conducted as a Post-Authorization Safety Study (PASS) requested by the Medicines Evaluation Board (MEB). Overall, 50,000 women were planned to be enrolled in the study. Recruitment started in 2009 and was finished in 2012. Follow-up of complete cohorts continued until 2014.
Participants/materials, setting, methods: A network of prescribing physicians enrolled women with a new COC prescription. During the follow-up phase, the woman was contacted regularly and asked for information about unintended pregnancies. Self-reported pregnancies were validated by health care professionals. Absolute numbers, incidence rates (per 10,000 WY), rate ratios, 95% confidence intervals and Pearl Indices were calculated. Inferential statistics were based on Cox proportional hazards models. Crude and adjusted hazard ratios between cohorts were calculated.
Main results and the role of chance: The final analysis is based on 50,203 participants, contributing 105,761 women-years (WY) of observation, thereof 30,098 participants and 73,174 WY in the European arm. European regulatory authorities were concerned about the low proportion of DNG/EV users in the United States (2%) and requested that the primary analysis should be based on the European study arm only. Mean age in the DNG/EV cohort was 31.9 (SD 10.0) and 25.7 (8.0) in the COC (other) cohort. In Europe, 226 unintended pregnancies were reported, of which 28 occurred under DNG/EV use, 192 under COC (other) use and 53 under COC (LNG). The Pearl Indices for the European DNG/EV cohorts were 0.3, and for the COC (other) cohorts 0.5 and for COC (LNG) users 0.8. Adjusted HRs (for age, parity, user status, smoking) of DNG/EV vs. COC (other) and vs. COC (LNG) for Europe were: 0.7 (95% CI: 0.5–1.0) and 0.5 (95% CI: 0.3–0.9). Stratification for pregnancies by compliance yielded Pearl Indices of 0.1 ("perfect HC use") and 0.3 ("non-perfect HC use") in the European cohort.
Limitations, reasons for caution: In the INAS-SCORE study, the number of participants in the US using DNG/EV were limited. The primary analysis is therefore based on the European arm of the study. Although the European DNG/EV cohort is older, age does not seem to have a strong effect on unintended pregnancies.
Wider implications of the findings: COCs have a high contraceptive effectiveness. Overall contraceptive failure rates were lower for the European DNG/EV cohort compared to LNG containing and other COCs.
Trial registration number: N/A.

O-208 Do subfertile women with *C. trachomatis* antibodies have reduced clinical pregnancy rates, a prospective 4-year follow-up study

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Study question: Is the past *Chlamydia trachomatis* infection measured by IgG and IgA antibodies responsible for reduced pregnancy rates in women with unexplained infertility?
Summary answer: Past *C. trachomatis* infection is not responsible for unexplained infertility, but time to successful pregnancy is longer with serologic marker of infection.
What is known already: A number of patients suffering from subfertility have evidence of past chlamydial infection as indicated by *C. trachomatis* antibodies (CAT) in serum. The association of tubal factor infertility and chlamydial infection has been widely recognized in several studies. It has also been suggested that achieving a clinical pregnancy spontaneously is lower among patients with positive Chlamydia serology, even without visible tubal pathology. Some studies have suggested the impact of past *C. trachomatis* infection on IVF success rates, whereas others have denied this correlation. Consequently, the impact of past *C. trachomatis* infection on unexplained infertility and pregnancy rates deserves further investigation.
Study design, size, duration: Our study is a prospective study consisting of subfertile couples referred for infertility investigations to the Department of Obstetrics and Gynecology, Helsinki University Central Hospital. During July 2007–December 2010, a total 228 women were enrolled for the study. The patients were followed from July 2007 to June 2014.

Participants/materials, setting, methods: The blood specimens of the participants were collected together with routine samples and further analyzed at the Institute for Health and Welfare in Oulu. Clinical data on participants as well as the results of their examinations, treatments and pregnancy rates were collected. *C. trachomatis* immunoglobulin A (IgA) and immunoglobulin G (IgG) antibodies were studied using commercially available EIA kits (AniLabSystems, Helsinki, Finland). Chi-squared test and Mann–Whitney *U*-test were used for statistical analysis.

Main results and the role of chance: Altogether 23.7% (54/228) of the women participants had antichlamydial antibodies, 5.7% (13/228) IgA and 19.7% (45/228) IgG antibodies, respectively. Women with antichlamydial IgA antibodies had history of miscarriages before seeking to infertility investigations more often than women who were seronegative (30.8 vs. 9.3%, $p = 0.036$). Surprisingly, the number of previous ectopic pregnancies did no differ between the groups. Spontaneous pregnancies occurred more often to women negative for chlamydial serology than to patients with positive markers of infection (36.5 vs. 21.4%, p = NS). Moreover, time to successful pregnancy was longer with patients with history of chlamydial infection measured by IgG antibodies than that of patients without (1.9 vs. 1.4 years, $p = 0.011$). Women with positive *C. trachomatis* antibodies did not suffer from unexplained infertility more often than the patients with negative serology. During the follow-up period, the number of miscarriages or ectopic pregnancies did not differ between the groups. In addition, IVF treatment was not needed more often among patients with positive Chlamydia serology.

Limitations, reasons for caution: The women were followed only in the Helsinki University Central Hospital area. Thus, we do not have information of patients moved to a different locality or were later treated in private clinics. Another limitation is that some women were conceived before the actual infertility examinations took place.

Wider implications of the findings: These results are useful in counselling subfertile women on their fertility prospects. In addition, the findings of this study can help us to plan the most suitable treatment for each couple and give guidelines whether to wait spontaneous fertilization or actively proceed to infertility treatment.

Trial registration number: X.

SELECTED ORAL COMMUNICATIONS

SESSION 56: ETHICS AND LAW

Tuesday 05 July 2016 **Room 101** **17:00–18:00**

O-209 The counsellor's advice: an analysis of moral implications of providing advice about how to disclose donor conception to children

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Study question: What are the moral implications of providing professional advice about the way to disclose a child's donor conception (DC) status?

Summary answer: Professional advice about family communication risks disempowerment of patients. Advice should be based on scientific evidence and its uptake by patients should be studied.

What is known already: Counselling is mostly directed at improving the patient's empowerment. A distinction is made between process advice and substantive advice. While the first is about teaching strategies for problem solving, the latter consists of specific suggestions, for instance, advice on how to respond to a child's questions and what tools (e.g., children's books) to use as an introduction.

Given the scarcity of follow up studies of donor conception families, little is known about how patients manage such advice in their daily lives. Examples of couples who apply advice in a stringent, counterproductive way can be found in the literature.

Study design, size, duration: The literature (including both theoretical and empirical research) was studied regarding ethics of counselling and advice giving, empowerment of patients and moral responsibility. Wide Reflective Equilibrium was used as a method to integrate normative and empirical insights.

Participants/materials, setting, methods: The starting point of the analysis are examples from published qualitative interviews with DC children and parents of DC children in which perception of professional advice as well as uptake of professional advice regarding communication about the child's DC status is discussed. Morally relevant factors are identified based on the literature.

Main results and the role of chance: Ethical standards of counselling describe process advice as morally unproblematic, in contrast to substantive advice. Our analysis shows several problems. First, in the perception of patients, a distinction between the two types of advice appears to be challenging. Second, we show that substantive advice or process advice that was perceived as substantive advice can be counterproductive or even harmful to the child and the family when applied in a decontextualized way: not taking into account the specific needs and features of the child or it's family. Third, instead of improving the self-efficacy and empowerment of parents, professional advice upholds the view that the professional (and not the patient) is the person who possesses the required knowledge and skills, thereby undermining the empowerment of patients. Fourth, patients may use the advice in an attempt to abdicate their moral responsibility for family communication.

Counsellors should avoid offering (perceived) substantive advice. Moreover, any advice offered should be based on scientific evidence. For both types of advice, counsellors have the professional obligation to gather evidence and facilitate research, including a follow up of how patients implement the advice and what the implications of such implementing are for all parties involved.

Limitations, reasons for caution: Patients who build their families using donor gametes often indicate that they lack scripts for communication about the DC. In that respect, they may expect and/or request advice. Withholding substantive advice does not mean that these needs should be ignored.

Wider implications of the findings: Because offering professional advice entails risks, counsellors have a professional responsibility to facilitate follow up studies on (the implications of) the uptake of their advice. Also in the debate about directive or non-directive counselling, the patients' perception of the counsellor's intentions should be studied as a relevant factor.

Trial registration number: The author is funded by the Special Research Fund of Ghent University. There are no competing interests.

O-210 Combination-PGD: ethics of access and transfer

W.J. Dondorp[1], V. Van der Schoot[2], C. De Die[2], G. De Wert[1]

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[2]*Maastricht University Medical Center, Department of Clinical Genetics, Maastricht, Netherlands*

Study question: Should accepted criteria both for access to PGD and for embryo-selection be widened in view of the growing number of requests for combination-PGD?

Summary answer: Criteria for access to PGD and for embryo-selection can be widened for secondary indications in combination-PGD.

What is known already: Professional guidelines reserve ICSI/PGD for those with a high risk of transmitting a serious disorder (or of repeated pregnancy loss due to a chromosome translocation). In several countries (NL, UK, Germany) legal instruments are in place to actually limit the scope of acceptable indications to such "high risk of serious harm" cases. With regard to transfer decisions, the corresponding rule is that only embryos without the targeted condition will be transferred. The ethical reasons behind these rules refer to societal costs, burdens & risks for the woman, the welfare of the child, and the moral sensitivity of embryo manipulation.

Study design, size, duration: Literature study, database research & ethical analysis. There is an increasing number of case reports of PGD treatments for two conditions. The focus of these publications is to emphasize the challenges and advanced technical procedures that this requires. Part of this literature refers to PGD plus aneuploidy testing (PGS), which is outside the scope of our study. Database research was done to determine the use of combination-PGD over the last 20 years in our country.

Participants/materials, setting, methods: Multidisciplinary desk research at academic PGD center. For the ethical analysis we used the method of "wide reflective equilibrium," which is an accepted approach in bioethics.

Main results and the role of chance: The literature and our data-base reveal that PGD is increasingly used for more than one genetic condition simultaneously.

As our ethical analysis shows, this has implications on several levels. First: as PGD is already done for a primary indication, there is no convincing reason for using the same strict access criteria for a secondary indication. However, the fact that a further cycle may more often be needed has proportionality implications that should be taken into account. Second: counseling for combination-PGD should address the possibility that no embryos unaffected by both conditions will be available and discuss the relevant wishes of the PGD-couple. Third, this requires transfer policy to clearly determine the conditions under which relevant options may be acceptable, including a further cycle or transferring embryos affected by a secondary condition that the procedure was initially meant to also rule out. The latter option implies a breach with the traditional rule of "never transfer an affected embryo." The primary ethical basis of this rule is the welfare of the child. However, to the extent that combination-PGD allows for less serious conditions to be accepted as secondary indications, transferring embryos affected by those conditions may well be acceptable.

Limitations, reasons for caution: The ethical implications of combination-PGD have not yet been systematically discussed in the literature. Our study is a first systematic contribution to this urgent debate.

Wider implications of the findings: Our conclusions are relevant for PGD policy making on all relevant levels (center policy, professional guidelines, health policy rules and regulations). For instance, in many countries access to PGD is bound to rules specifying strict access criteria that do not take account of the ethically relevant aspects of combination-PGD.

Trial registration number: N/A.

O-211 Preimplantation genetic screening (PGS): a blessing or a curse for patient centred fertility treatment?

H. Mertes[1]
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Study question: Under which circumstances would PGS (preimplantation genetic screening) benefit, rather than threaten patient centred fertility treatment?

Summary answer: As the (potential) utility of PGS varies according to one's definition of successful treatment, patient education and joint decision making will be of utmost importance.

What is known already: There is currently much controversy over whether or not PGS should (already) be introduced into the clinic and if so, for which patient population. Scientific evidence is currently being gathered in order to establish the benefits and drawbacks of this new technology. Important outcome measures will be cumulative pregnancy rate per cycle started, time to pregnancy, miscarriage rate (all three for different age ranges), health outcomes for the offspring and cost-effectiveness of the treatment. Expectations are that PGS will lower the cumulative pregnancy rate, but also shorten the time to pregnancy and reduce miscarriage rates.

Study design, size, duration: A philosophical assessment was made about the most ethical way of introducing PGS into the clinic, starting from the premise that it will deliver on its promise of predicting the implantation potential of the *in vitro* embryo. The method that is used to bring empirical data (as found in literature research) and normative ethics together is the "Wide Reflective Equilibrium," the most commonly used method in bioethics.

Participants/materials, setting, methods: Claims about the benefits and drawbacks of PGS (Anno, 2015) as found in scientific literature, position statements of professional societies and commercial marketing were gathered and assessed for validity against current evidence. In light of patient-centred care, the relevance of these claims on burden, effectiveness, safety and financial cost was assessed. This information was then coupled with the treatment outcome measures that patients deem important (as found in the literature) and scanned for possible misconceptions.

Main results and the role of chance: Currently, most of the controversy over PGS is focussing on whether or not (or to what extent) this technology is successful at predicting which embryos will lead to a healthy live birth. However, proving that PGS "works" does not prove that it will benefit the individual patient.

PGS is often marketed as a technology that increases IVF success rates. This success rate certainly does not refer to the "singleton, term gestation, live birth rate per cycle initiated" as PGS can never increase this rate. Rather, it refers to a reduction in miscarriage rate and time to pregnancy and/or to an increase in implantation rate in the first transfer cycle. If and when this treatment option is offered to patients, it is therefore of utmost importance to know what the patient's definition of success is. For example, if the psychological burden of repeated miscarriages is unbearable for patient 1, while the thought of risking the loss of a viable embryo in the last stimulation cycle she can afford is unbearable for patient 2, PGS might benefit patient 1, but not patient 2. More attention should go to cost-benefit analysis and to managing misconceptions and unrealistic expectations.

Limitations, reasons for caution: An assessment of *how* PGS should be introduced into the clinic does not imply that the debate about *whether* we need PGS or whether it can fairly accurately predict which embryo will lead to a live birth is settled.

Wider implications of the findings: Fertility clinics need to be aware that whether or not a certain new technology is beneficial to a patient cannot be decided based on scientific evidence alone, as it depends on the patient's personal values and personal definition of success.

Trial registration number: N/A.

O-212 Genetic conditions in gamete donors and the handling of genetic risk

L. Bruhn Madsen[1], O. Schou[1]
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Study question: All humans carry several genetic diseases and are predisposed for various genetic conditions. How should this issue be addressed without excluding all gamete donors from donor programs?

Summary answer: A platform has been developed considering the "right not to know" which simultaneously reveals genetic conditions allowing for the use of donors with genetic conditions.

What is known already: Genetic screening of sperm donors is an important component in the selection of gamete donors. It is a fact that every human is a carrier of several recessive genetic diseases, but guidelines presenting a uniform recommendation on how to address this issue in a donor perspective are still lacking. Current recommendations for genetic screening of gamete donors mainly consist of a medical history of the donor's family and testing for a limited number of frequent recessive disorders. Although this decreases the risk for transmitting certain genetic diseases through donor conception, the likelihood for transmitting genetic disorders can not be avoided.

Study design, size, duration: During the last decades there has been an increase in the number of reported conditions on gamete donors even though donor screening programs are more thorough than ever. This means, that currently approximately 20% of all donors at the sperm bank Cryos International, who have passed initial screening procedures, have subsequently been associated with a genetic condition mainly of multifactorial or recessive nature. This figure will continue to increase since all donors carry genetic diseases.

Participants/materials, setting, methods: Donors with known genetic conditions identified through adverse reaction reports are made available in a transparent system hosted by Cryos International allowing for the recipients to make an informed choice with respect to the usage of specific donors.

Main results and the role of chance: To accommodate for the fact that everyone carries recessive diseases and genetic variants predisposing for multifactorial diseases a platform has been developed where knowledge on donors with registered genetic conditions are made available. This serves the purpose of transparency and also that sperm from donors with registered genetic conditions are made available for distribution. However, not everyone wants to know, and therefore the platform has been developed to acknowledge the "right not to know," which also means that several steps have to be actively accomplished before specific knowledge is made available.

Despite web-based efforts, and subsequent e-mails to make sure that recipients actually understand that their chosen gamete donor has been registered with a genetic condition, assessment of the distribution of sperm from the donors reveals that it is still employed beyond the usage for siblings, although to a lesser extent than sperm from donors with no registered conditions. Still, this indicates that there might be a movement away from the illusion of preventative perfectionism towards an acceptance of the fact that avoiding all risk is impossible, which might even form the basis for expanded genetic screening programs allowing for inclusion of donors, where initial genetic screening has revealed heterozygosity.

Limitations, reasons for caution: Although individuals are explicitly informed when using the system, and have to give consent before genetic information is

disclosed, there is still a possibility that the achieved information is not cognized, which is why professional guidance is recommended prior to the use of gamete donors with known genetic conditions.

Wider implications of the findings: This abstract contributes to the discussion about the range of genetic screening of gamete donors and the need for harmonization of guidelines for genetic screening and how to assess genetic risk in gamete donors.

Trial registration number: N/A.

INVITED SESSION

SESSION 57: MOLECULAR ELEMENTS IN MALE INFERTILITY

Wednesday 06 July 2016 **Hall 1** **08:30–09:30**

O-213 Male infertility as a monogenic pathology

S. Viville[1]

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Despite 38 years of IVF practice, human gametogenesis remains a black box and many of the molecular mechanisms underlying this process remain to be discovered. This lack of knowledge is mainly due to the absence of an *in vitro* model for human gametogenesis. The direct clinical consequence is the large number of patients for whom no diagnosis is available. Indeed, the cause of infertility in about half of the couples seeking reproductive assistance remains idiopathic. For many of them infertility has a genetic origin. During the last decade, a large effort was undertaken to identify genes that are solely involved in human infertility. Following this effort the number of "infertility genes" grew very quickly. Such research has many rewards. In terms of basic science, it is a powerful strategy to identify gametogenic molecular pathways and to study them with the help of appropriate models. A better understanding of human gametogenesis will bring new tools not only at the diagnostic level but also for the development of new reproductive technologies. At the clinical level, it opens the possibility to offer proper diagnosis and to determine the prognosis and the best management of such couples, therefore providing the most adapted therapeutics and counselling strategies to them.

During my presentation, I shall give a quick overview of the genetic causes of human infertility. These include numerical or structural chromosomal anomalies, syndromic and non-syndromic genetic pathologies. In addition, I shall mention the few studies, so called "whole genome association studies," which have been performed to try to establish correlations between genetic makers and human infertility. These try, not so successfully, to correlate infertility phenotypes with genetic markers. The major limitation of these studies resides in the phenotypic heterogeneity and the limited number of patients and controls analyzed.

Chromosomal anomalies have been well known for a long time and karyotype analyses have been well established in the reproductive field. Most of the syndromic pathologies are too severe to allow patients enough time and/or energy to set up a reproductive project. For non-syndromic genetic infertility, during the last decade, studying family cases of infertility or groups of infertile patients, the list of human genes has grown very quickly. So far, there are nearly 20 genes for which data are firm enough to consider them as responsible for a non-syndromic infertility phenotype in humans when mutated. The fact that over 200 infertile or subfertile genetic mouse models have been described gives an idea of the long pathway still to go in human studies to identify genes that could be involved in male and female reproduction. It also pinpoints the high degree of complexity of the gametogenesis process. I shall describe the strategies used to identify these genes, which should give you an understanding of how you could participate in this research effort. I shall also give some examples to underline the importance of studying these genes in order to better understand the human gametogenic process.

Last, I shall share some recommendations of what to offer your patients and some perspectives.

O-214 Transposable elements and male gametogenesis

P. Fauque[1], D. Bourc'his[2]

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The epigenetic setting of the germline is known to be important for the acquisition of genomic imprinting, through the deposition of sex-specific methylation marks that will lead to the differential expression of paternally and maternally inherited alleles of a handset of genes after fertilization. Perturbation in imprinting acquisition in gametes has well-recognized consequences on the occurrence of epigenetic disorders, such as Beckwith–Wiedemann syndrome (Robertson, 2005).

Other targets of the germline-specific epigenetic reprogramming are transposable elements (TEs). TEs make up the majority of the genetic material. Indeed, the release of the human genome sequence has revealed that TEs represent more than half of genomic mass. This transposon landscape reflects an evolutionary tug-of-war between integration and propagation events orchestrated by these elements, and counteracting defense mechanisms exerted by the host. For genetic innovation and diversification, transposons have participated to genome shaping and speciation during evolution. However, on a short-term basis, transposons can adversely modify the function and the architecture of the genome (Ostertag and Kazazian, 2001). Spurious relaxation of transposon control can allow mobilization of these elements, which can disrupt genes by insertional mutagenesis. When occurring in somatic cells, cancer can ensue; when occurring in gametes, heritable mutations can be generated and lead to congenital disorders (Cordaux and Batzer, 2009).

Recent studies have highlighted the existence of specialized pathways acting in the germline for the life-long repression of transposons and the protection of the hereditary material (Zamudio and Bourc'his, 2010). Genetic impairment of this system in mice invariably leads to massive transposon reactivation and complete failure to produce spermatozoa in males (Bourc'his and Bestor, 2004). Transposon control therefore appears as a major safe guarder of male fertility in mouse, but also in other animal species. Such a role is highly likely to be conserved in human. The purpose of this lecture is to shed light onto these newly appreciated determinants of male reproductive fitness, the transposons. Improper control of these elements, of genetic or environmental origin, could be revealed as a major cause of male infertility (Fauque and Bourc'his, 2014).

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INVITED SESSION

SESSION 58: ENDOMETRIAL INJURY PRIOR TO EMBRYO TRANSFER IN IVF

Wednesday 06 July 2016 **Hall 5 CB** **08:30–09:30**

O-215 Does it make scientific sense?

H. Critchley[1]

[1]*University of Edinburgh, The Queen's Medical Research Institute, MRC Centre for Reproductive Health, Edinburgh, UK*

Successful implantation is a multi-step process that requires synchronous development of both the endometrium and embryo. Implantation is a rate-limiting step in assisted reproduction and a mismatch between endometrial and embryo development may play a role in implantation failure. When a pregnancy fails to occur there is an equally exquisitely, spatial and temporally, regulated physiological breakdown, shedding and repair of an "injured/wounded" endometrium (menstruation and endometrial repair). Underpinning this physiological event of injury and repair are hormone withdrawal, local tissue hypoxia, cyto/chemokine production and leukocyte traffic.

Endometrial "scratching"/"injury" is intended local damage to the endometrium that has been reported to have impact on subsequent receptivity. To

date there is however no consistent mode of "injury": biopsy or curette; depth or number of injury events; cycle phase for imposing injury event; interval between injury and treatment cycle. Furthermore there is an endometrial breakdown and repair event between the act of injury/trauma and the subsequent cycle which prepares to be receptive for a potential implantation.

The endometrium is a complex, steroid-dependent tissue. Its form and function reflect exposure of component stromal, epithelial, vascular and immune cells, in both the superficial and basal layers, to endocrine and intracrine steroid signals (oestrogen, progesterone, androgen, glucocorticoids). Endometrial receptivity involves a transformation of the stromal compartment and tightly co-ordinated stromal-epithelial cross-talk. The peri-implantation milieu also involves immune cells and cytokine production, in turn regulated by the prevailing hormone environment.

It has been suggested that a local "mechanical" endometrial injury may provoke decidualisation; an inflammatory response, and/or a vascular reaction; and delay endometrial maturation and thereby, in some way, enhance preparation of the endometrium for implantation in the next menstrual cycle. The molecular/cellular mechanisms by which "endometrial scratch"/local endometrial injury may potentially lead to improvement in IVF outcome in women still remains unclear. The molecular and cellular mechanisms that underpin injury-induced events in the endometrium (physiological and mechanical) will be considered.

O-216 Does it make clinical sense?

C. Blockeel[1]
[1]*UZ Brussel, Centre for Reproductive Medicine, Jette, Brussels, Belgium*

Despite the vast development in assisted reproductive technologies (ART) during the last 30 years, live birth rates have remained low and, since the year 2000, rather stagnant. Researchers have postulated that endometrial non-receptivity may now be the main reason why even morphologically top quality embryos still frequently fail to implant.

The intricate dialogue between the embryo and endometrium is generally only possible during a specific timeframe of the secretory phase – the window of implantation. Previous studies have shown that there is a disruption of the natural endocrine function during ART caused by exogenous ovarian simulation, potentially hindering endometrial decidualization, function and receptivity.

Intentional endometrial injury, also referred to as "endometrial scratching," is one among many adjuvant methods designed to enhance implantation during ART. This technique, first described in the animal model by Loeb in 1907, seems to potentially improve receptivity, albeit by unknown mechanisms.

Evidence deriving from randomized controlled trials, systematic reviews and meta-analyses moderately favour inducing local injury in women with recurrent implantation failure. However, these results have been a subject of much debate, with detractors pointing out methodological weaknesses in the presented data and a general lack of basic scientific evidence to prove a definite beneficial effect of endometrial scratching. Furthermore, the heterogeneity within the clinical trials performed thus far have further increased the controversy, since the method and timing of the endometrial scratching have varied greatly. Nonetheless, owing to the fact that endometrial scratching is so easy to use in daily clinical practice, many physicians have hastily applied this technique unselectively to their patients in all fields of reproductive medicine, including intrauterine insemination and frozen embryo transfers. This widespread use has generated the perfect storm which has pitted the proponents and the detractors of this technique even further from each other and from the current scientific knowledge all-together.

In this presentation, a review of the current evidence, gaps of knowledge and future prospects regarding the potential benefit of endometrial scratching will be provided.

INVITED SESSION

SESSION 59: PARAMEDICAL INVITED SESSION - CONTROVERSIES - "EMBRYO TRANSFER EXCLUSIVELY IN THE FROZEN CYCLE?"

Wednesday 06 July 2016 **Room 101** **08:30–09:30**

O-217 Does it increase the probability of pregnancy?

B. Shapiro[1]
[1]*Fertility Center of Las Vegas, Las Vegas, NV, USA*

Fresh embryo transfer was the default treatment for women undergoing *in vitro* fertilization (IVF) for almost 40 years. Recently, however, there has been increased use of embryo banking cycles, in which all embryos are cryopreserved for subsequent thaw and transfer in a later cycle. The increasing use of embryo banking may be for any of several purposes. These include reducing the risk of ovarian hyperstimulation syndrome (OHSS), suspending embryo development while genetic test results are obtained, and postponing embryo transfer to obtain a better uterine environment in the absence of uterine exposure to controlled ovarian stimulation (COS). It has been shown that the implantation rate, clinical pregnancy rate per transfer, and ongoing pregnancy rate per transfer are increased with frozen-thawed embryo transfer (FET) into an artificially prepared endometrium when compared to fresh embryo transfer following exposure to COS. This was shown in a randomized trial in a population of normal responders undergoing their first IVF cycle.

Endometrial receptivity can be impaired following COS, primarily through an effect of embryo-endometrium asynchrony. Numerous clinical and basic science studies have revealed advanced endometrial histology and advanced down-regulation of the progesterone receptor following COS exposure. It has been shown that slowly developing embryos (day 6 blastocysts) are less likely to implant that their faster counterparts (day 5 blastocysts) if transferred in fresh cycles following COS exposure. This relationship diminishes or vanishes in cycles lacking COS exposure, such as oocyte donation and transfers of frozen-thawed embryos after artificial endometrial preparation. Despite the potential for cryo-damage, thawed day 6 blastocysts have been repeatedly shown to implant more readily than fresh day 6 blastocysts.

The evidence for impaired endometrial receptivity is much less clear in high responders. Two reports have concluded no significant evidence of impaired receptivity in high responders, although one was halted early for safety reasons. It seems high responders are more likely to have at least one rapidly developing embryo and therefore are more likely to have good embryo-endometrium synchrony.

However, the benefits of cryopreservation have a price. Some embryos do not survive thaw, and some transfers are therefore cancelled. The risk of embryo loss must be balanced against the improved endometrial receptivity. This balance might vary depending on methodology and patient characteristics.

Also, the benefits described above are per transfer, not per retrieval. This is a particular concern if post-thaw non-survival is a significant cause of cancellations. A meta-analysis of three randomized trials comparing FET and fresh transfer concluded the ongoing pregnancy rate per retrieval (through the first transfer) was greater in patients randomized to FET than in those randomized to fresh transfer. However, the largest study in that meta-analysis was subsequently withdrawn.

Another important measure is the cumulative success rate per retrieval, considering all transfers from each embryo cohort. Our own results, not previously published, reveal no significant difference in cumulative live birth rate per retrieval between patients randomly assigned to either fresh transfer or FET, after subsequent transfers of frozen-thawed supernumerary embryos were considered in both groups. In this follow-on analysis of the combined results following two randomized trials, the cumulative live birth rate is now final, because no patient who failed to have a live birth still has any remaining frozen supernumerary embryos. However, both studies employed only conventional slow-freezing, potentially reducing cumulative success rates when compared to current vitrification techniques.

Fresh autologous transfers can achieve excellent success rates in selected circumstances, specifically when embryo development is rapid (day 5 blastocyst expansion) and pre-ovulatory progesterone levels are low.

Future research should compare success rates per retrieval in patients randomized to fresh or vitrified blastocyst transfer using the most current techniques.

O-218 Does it affect the mother and the offspring?

S. Bhattacharya[1]
[1]*University of Aberdeen, Obstetrics and Gynaecology, Aberdeen, UK*

In vitro fertilisation (IVF) has traditionally tended to rely on a strategy of transferring the best quality fresh embryo or embryos within the endometrial cavity during the treatment cycle. Increasing success rates associated with embryo cryopreservation have allowed spare embryos are now usually frozen and replaced later – either in natural or hormonally manipulated cycles. High levels of

estrogen and progesterone produced during ovarian stimulation could result in a relatively hostile intrauterine environment whilst increasing the risk of ovarian hyperstimulation. Electively freezing embryos with the intention of thawing and replacing them within the uterus at a later stage when the influence of supra-physiological levels of ovarian steroids have abated, could increase pregnancy rates and reduce complications.

Data from two small randomised trials suggest potentially better pregnancy rates following elective frozen embryo transfer but have not been able to provide robust data on perinatal outcomes. Analyses of large observational datasets suggest that the risks of preterm birth, small for gestational age, antepartum haemorrhage are reduced in pregnancies following frozen embryo transfer but these results are not free from serious bias and confounding. Some women have contributed more than one pregnancy to the dataset and it is not always been possible to adjust for key variable such as parity, body mass index and smoking. Thus, the existing evidence base is insufficient in terms of quality to warrant a major change in clinical practice towards a strategy of elective embryo freezing in all couples. In addition some recent studies have demonstrated an enhanced risk of large for gestation al age associated with frozen embryo transfer, which needs further evaluation. Large adequately powered pragmatic randomised trials are urgently needed. Follow up of offspring conceived by women randomised to frozen embryo transfer is the best way of evaluating perinatal outcomes.

SELECTED ORAL COMMUNICATIONS

SESSION 60: EMBRYO TIME-LAPSE MICROSCOPY

Wednesday 06 July 2016 **Hall 1** **10:00–11:45**

O-219 Time lapse technology allows us to study the effect of sperm quality in the embryonic development

D. Agudo[1], G. Pirastu[1], D. Cernuda[1], F. Bronet[1]
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Study question: Is embryo kinetics affected by sperm quality?
Summary answer: Our data show a direct cleavages increase and a cell division synchrony decrease in microinjected embryos with oligoasthenoteratozoospermics spermatozoa.
What is known already: Time lapse technology is offering a multitude data about kinetics of embryonic development. In addition, some studies have tested whether embryonic kinetics is affected by culture medium, ovarian stimulation, oxygen tension, origin of the eggs, the age of the patient, but few have studied influence of the male gamete in embryonic kinetics.
Study design, size, duration: Retrospective study of 118 oocyte donation cycles. Two groups of patients were compared. 80 cycles (863 oocytes, 719 embryos) were good prognosis patients sperm samples (more than 15 million/ml, more than 30% of motility, more than 4% of normal forms) (group A) and 38 cycles (427 oocytes, 303 embryos) coming from poor prognosis sperm samples (less than 5 million/ml, less than 5% of motility and less than 1% of normal forms) (group B).
Participants/materials, setting, methods: This cycles were incubated in embryoscope. We studied Cellular events described by Meseguer et al. (2011), including all cellular divisions until blastocyst stage, appearance and fading of some cellular structures, and two cellular events described as cc2 (difference in hours between first and second cellular cleavage) and S2 (difference in hours between second and third cellular cleavage). Data were exported from the embryo viewer data base. SPSS software was used on data analysis.
Main results and the role of chance: No significant differences between two groups under study (A and B) were found respecting the time of cell division (T2, T3, T4...). However when percentage of embryos, that had synchrony in the range S2, were analyzed (less than 5 h) we found statistically significant differences ($p = 0.0087$), when comparing both groups (46.94% in A group and 37.42% in group B).

Moreover, we subdivided groups A and B into two subgroups respectively. The first subgroup consists of transferred and frozen embryos, and the second consists of discarded embryos. We found statistically significant association in every time of cell division, when we compared the two subgroups of the group A, observing a development delay in discarded embryos. However no differences were found between the group B two subgroups in T3, T5, T9 and s2 times, concurring with second, third and fourth cell cycle starting times, which might reflect a delay in cellular interphase.
Limitations, reasons for caution: This study has been performed in oocyte donors, which form a fairly homogeneous group in terms of age and ovarian response, so these results may not be extrapolated to other groups of women undergoing an assisted reproductive treatment. However, more data are needed to draw firm conclusions.
Wider implications of the findings: The sperm sample quality may affect the synchrony in second cell cycle (S2) and may induce a delay in second, third and fourth cell cycle in embryos transferred and frozen coming from poor quality sperm samples if we compare to embryos discarded.
Trial registration number: 1406-MAD-047-DA/NCT02155179 Clinical trials/COI: MAD-DA-09-2014-05.

O-220 Cinematographic analysis of embryo vacuoles and their impact on reproductive outcome

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Study question: To evaluate the dynamics of the impact of spontaneous appearance of vacuoles in embryo quality and clinical outcome after time-lapse evaluation with donor oocytes.
Summary answer: No significant, although relevant differences, were found in the number of good quality embryos, pregnancy and implantation rates between non-vacuolated (NVE) and vacuolated embryos (VE).
What is known already: In animal cells, vacuoles are implicated in transport and cellular digestion processes. The spontaneous appearance of vacuoles in oocytes and embryos in IVF cycles is relatively frequent. Although this morphological aspect is considered detrimental for embryo outcome, the conclusions derived from bibliography are controversial and are based in single point morphological observations. It is accepted that little vacuoles (≤5 µm) in less than a half of the embryo are better than bigger ones in many cells. However, the use of video-time lapse has shown that vacuoles are a very dynamic process in terms of time of appearance, number and size.
Study design, size, duration: Retrospective study of 340 embryo transfer (either fresh or cryopreserved) at blastocyst stage, from our oocyte donation program, performed between October 2011 and December 2014. A total of 1354 embryos were analyzed, from which 499 blastocyst were transferred. 54.3% of the embryos presented at least a vacuole along the development. For implantation analysis, only embryos with Known Implantation Data (KID) where considered ($n = 376$). 223 of the 376 KID embryos were vacuolated (59.3%).
Participants/materials, setting, methods: Ovarian stimulation was performed with rFSH and GnRH antagonist. Ovulation was triggered with GrRH agonist.

After ICSI, oocytes and embryos were cultured in continuous medium (Global, Life-Global) at 37°C, 5% CO_2, 5% O_2, in Embryoscope™.

Morphological and kinetics events of embryo development were recorded and analyzed in the EmbryoViewer™. Appearance of vacuoles was matched in each stage, from oocyte to blastocyst.

Embryo quality, pregnancy and implantation were compared between NVE and VE.
Main results and the role of chance: The first spontaneous appearance of vacuoles was more frequent: between the extrusion of de 2° Polar Body and 2 cells (31% in all studied embryos and 29% in the transferred blastocyst), and especially from 8 cells to Morula stage (M) (54.3% in all studied embryos and 58.1% in the transferred blastocyst).

There were no differences in terms of good quality blastocysts between NVE [72.4%, (95% CI 68.2–76.5)] and VE [74.2% (95% CI 70.8–77.6)] ($p = 0.49$).

There were significantly fewer arrested VE than NVE [18.8%, (95% CI 15.9–21.6) vs. 37.2% (95% CI 33.4–41), $p < 0.0001$].

There were no significant differences in terms of clinical pregnancy [60.2% (95% CI 50.7–69.6) vs. 49.7% (95% CI 42.3–57.1), $p = 0.09$], and implantation rate [49.7% (95% CI 41.7–57.6) vs. 39.9% (95% CI 33.5–46.3), $p = 0.06$]

between NVE and VE, although clinical outcome decreases in 10 points approximately.
Regarding to KID VE, the better the embryo quality is, the better implants: There are significantly more high quality embryos in the group of implanted VE (48.3 vs. 37.6%, $p = 0.002$) than in the non-implanted one.
Limitations, reasons for caution: Spontaneous vacuolization may be modified by oocyte and embryo characteristics and culture conditions. Taking into account the dynamic behavior of this event, the annotation of vacuoles were made just before the next embryo cleavage.
Wider implications of the findings: Appearance of vacuoles does not affect embryo quality or arrest. We found a relevant, although not significant, decrease in pregnancy and implantation when VE are transferred, due to the sample size. Good quality VE implant better than low quality ones. VE should be a second alternative for embryo transfer.
Trial registration number: None.

O-221 Morphokinetic analysis of early fertilization and embryo cleavage dynamics based on the mode of insemination technique used on human embryos: a prospective sibling oocyte study

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Study question: Does the mode of insemination create disturbances in embryo developmental timings in couples undergoing assisted reproductive treatment (ART) with their own gamete cells?
Summary answer: Both insemination method showed similar morhokinetic patterns for all the other timepoints compared, except that IVF embryos reached expanded blastocyst-stage (tEB) significantly faster.
What is known already: ART registry data in many countries as well as international societies state that the use of ICSI for other than severe male infertility is on the rise. Since it bypasses many biological events in the course of fertilization, there always exist a debate regarding whether the use of ICSI *per se* can increase the risk of chromosomal or congenital abnormalities or not. Recent introduction of time lapse analyses start to bring valuable information regarding embryo dynamics and the studies using this technology in order to delineate the contribution of the fertilization method in the laboratory & clinical outcome is scarce.
Study design, size, duration: This prospective sibling oocyte study was performed in Bahçeci Fulya IVF Centre between April 2014 and October 2015. It includes cycles having female age less than 38, with sperm concentration >15 million/ml, motility >50% and morphology >4%.
Participants/materials, setting, methods: A total of 37 cycles were included. For each cycle, retrieved oocytes were randomly and equally assigned into IVF and ICSI during ART treatment and simultaneously processed for IVF (Group IVF) or ICSI (Group ICSI). Fertilized oocytes were cultured in a time lapse incubator and scored for morphokinetic check points (time points from tPNf through tHB) until embryo transfer or cryopreservation.
Main results and the role of chance: Overall, 780 oocytes were found to be at M2 stage and inseminated according to study protocol. Fertilization rates for IVF and ICSI groups were 75.8 and 80.8% respectively. Based on the capacity of the time lapse incubator, 406 of these fertilized oocytes (195 by IVF and 211 by ICSI) were simultaneously cultured and scored for morphokinetic parameters from pronuclear fading until blastocyst stage. When tPNf is taken as t_0 for comparative analysis, embryos on both arms of the study showed similar embryo cleavage dynamics as well as quality. The only significant difference between the study groups was found to be the time to reach expanded blastocyst-stage (tEB). Embryos generated after IVF were found to reach to the expanded blastocyst stage significantly earlier than that of ICSI-fertilized embryos ($p < 0.02$).
Limitations, reasons for caution: This study includes an isolated group of infertile cases in which both oocyte and sperm quality are devoid of any known morphological abnormalities. Our results should therefore be taken as preliminary when they are extrapolated for cases with lower oocyte & sperm yield/quality.
Wider implications of the findings: Our results can show that the insemination technique *per se* does not create significant distortions on the morphokinetic behavior of the *in vitro* fertilized embryos. The differences observed in the literature can therefore be due to the intrinsic characteristics of the gamete cells from which they were produced.
Trial registration number: None.

O-222 Euploid blastocysts that experience more collapses are less likely to result in live birth: a quantitative and automated analysis of time-lapse cinematography

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Study question: What are the dynamic blastocyst collapse patterns between euploid blastocysts that do or do not result in a live birth?
Summary answer: Euploid blastocysts that experienced higher number of collapses and more frequent collapses were less likely to achieve live birth.
What is known already**:** Pre-implantation genetic screening (PGS) improves IVF success by selecting against aneuploid embryos. Morphology has been shown to correlate with blastocyst aneuploidy, but not with implantation of euploid blastocysts. Currently effective embryo assessment methods that help selection among euploid blastocysts are highly desired to further enhance the success rates of euploid embryo transfer. Blastocyst collapses have been found to associate with lower likelihood to hatch and/or implant in animal and human studies. This study examined the relationship between blastocyst collapse patterns and euploid embryos resulting in live birth.
Study design, size, duration: Prospective observational study. Of 102 patients enrolled (August 2012–November 2013), 32 had Day 3 laser-assisted hatching and were excluded. Of the remaining 70 patients, 11 had no transfer and 4 had unavailable image analysis. Therefore 55 patients with 85 transferred euploid blastocysts from fresh/frozen cycles were analyzed. Four transferred embryos were further excluded as they expanded <10% on Day 5 (one led to live birth), and the final dataset was comprised of eighty-one embryos (forty-three led to live birth).
Participants/materials, setting, methods: Entire cohorts of embryos from patients were imaged using the Eeva System. Time-lapse images were automatically processed to extract dynamic parameters including number, frequency, time, degree and rates of collapses. Median values for the parameters were reported, and Mann–Whitney–Wilcoxon Test was performed to calculate *p*-values. Following qPCR-based comprehensive chromosome screening, euploid blastocysts were selected for transfer and patients were followed until delivery, with DNA finger printing performed when necessary to determine outcome of individual embryos.
Main results and the role of chance: A fully automatic image analysis routine was developed to calculate the size of the embryo for each frame of the video (one frame every 5 min). The computer automated embryo size calculation was validated for its accuracy by comparing to manual measurements (96%). Dynamic profiles of blastocyst size vs. time were then automatically analyzed by MatLab programs to identify each collapse event and output the timing and embryo size at the start and end of each collapse event. Euploid blastocysts that resulted in live birth experienced a 62% relative decrease in collapse events compared to those that resulted in no live birth (median 4 vs. 6.5, $p = 0.008$). Euploid blastocysts selected for transfer had an average collapse frequency of 0.37 ± 0.27 collapse events per hour. Euploid blastocysts that resulted in live birth had statistically significantly lower frequency of collapse events (median 0.30 vs. 0.43 collapse events per hour, $p = 0.004$). Consistent with the findings above, euploid blastocysts that resulted in live birth spent significantly less time during the collapse phase (11 vs. 15%, $p = 0.002$).
Limitations, reasons for caution: This is an observational study performed in a single centre, and therefore the clinical significance of the finding needs to be validated in a multi-centered study with a larger sample size.
Wider implications of the findings: This study is the first to examine the relationship of automatically-measured human blastocyst collapse dynamics with live-birth. Euploid blastocysts with more frequent collapses appear to have a lower live-birth rate, suggesting that frequent collapses may compromise embryo viability. Our findings may assist embryo selection for PGS-patients with multiple euploid embryos.
Trial registration number: Clinicaltrials.gov, NCT01635049.

O-223 Pregnancy outcomes of single cleavage-stage embryo transfer by using Time-lapse imaging do not differ from single blastocyst transfer

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Study question: By using time-lapse imaging (TLI), whether single cleavage-stage embryo transfer (SCT) on Day 3 can achieve the same pregnancy outcomes as single blastocyst transfer (SBT) on Day 5?
Summary answer: SCT achieved the same pregnancy outcomes as SBT by TLI. No significant differences were found in implantation rate and early abortion rate between two groups.
What is known already: TLI is a powerful tool for selecting competent embryos, which enables embryo development to be monitored continuously. SBT has been advocated to eliminate multiple births while a high pregnancy rate is guaranteed. However, SBT increases the risk of cancelled cycles, epigenetic disorders and preterm delivery due to a long culture time.
Study design, size, duration: A retrospective study was performed from January 2014 to December 2014 for establishing the embryo selection criteria using TLI. A prospective randomized study of the SET on Day 3/5 was conducted by using the established criteria during January 2015 to November 2015. When the optimal embryos ≥3 per cycle on Day 3, women were randomly assigned to SCT Group or SBT Group (If the patient refused to SBT, this cycle would not be included).
Participants/materials, setting, methods: A total of 1355 embryos were used for blastocyst culture in the reprospective study. The time of pronuclear fading (PNf) was defined as the starting point. During 104 SET cycles, SCT was conducted in 65 women and SBT was conducted in 39 women. Logistic regression analysis was used to evaluate the association of embryo cleavage patterns, timing parameters and blastocyst quality. Pregnancy outcomes were analyzed using the Chi-square test.
Main results and the role of chance: A new criteria was established for selecting the optimal embryo for SET in which embryos with normal cleavage, uneven cleavage or non-axial cleavage patterns simultaneously had optimal time intervals [*s*2 (synchrony of the second cell cycle) ≤ 0.5 h and *t*5 (five cells time point) ≤ 26.67 h)]. No significant difference was found in terms of the implantation rate (64.62 vs. 66.70%) and early abortion rate (4.44 vs. 3.85%) between SCT group and SBT group.
Limitations, reasons for caution: Large-scale clinical trials of SET on Day 3/5 with take-home infant rates as the endpoint were required to further prove the effectiveness and safety of SCT.
Wider implications of the findings: The use of the new criteria would improve the selection of embryo with better development potential. This process can improve the implantation rate of SCT, thus to further promote SET, in order to reduce multiple pregnancy rate and culture time.
Trial registration number: The study was registered at ClinicalTrial.gov with accession number NCT02313311.

O-224 Time-lapse algorithms and morphological selection of blastocysts for transfer: a pre-clinical validation study

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Study question: What is the agreement between published time-lapse algorithms in selecting a day-5 embryo for transfer and what is the agreement between these algorithms and embryologists?
Summary answer: The agreement between published time-lapse algorithms was surprisingly variable while it was only fair between these algorithms and embryologists.
What is known already: The introduction of time-lapse incubators has allowed for the provision of more information regarding embryo growth and development. The timing and duration of embryo developmental events has been correlated with pregnancy, and allowed the developed time-lapse algorithms to select an embryo for transfer with presumably the highest implantation potential. However, the generalizability of these algorithms still remains highly questionable, and given that randomized controlled trials (RCTs) are costly and demanding in resources, it might be useful to firstly assess the pre-clinical validity of these algorithms in a different setting before conducting a RCT.
Study design, size, duration: This was a prospective study (n = 100 cases with ≥2 day-5 embryos cultured in the EmbryoScope) comparing the agreement in selecting the best day 5 embryo for transfer between published time-lapse algorithms aiming to improve implantation or pregnancy rates. Furthermore, the agreement between these algorithms and 10 embryologists at five different clinics was assessed. The cases included were from a pre-clinical phase prior to EmbryoScope use, where embryologists did not apply any algorithm during embryo selection.
Participants/materials, setting, methods: Three published algorithms were identified and analyzed in this study. (A) Meseguer et al. (2011), (B) Basile et al. (2015) and (C) Goodman et al. (2015). Embryologists were also asked to choose an embryo for transfer using 2D images. For each case, day-5 images were provided, followed by a day-3 and day-5 image of the same embryo. Agreement between the algorithms as well as the algorithms and the embryologists was assessed using the kappa coefficient.
Main results and the role of chance: The mean number of day-5 embryos available was 4.28 (standard deviation-SD: 1.99). Application of algorithm A, B and C resulted in 63, 53 and 53% of cases having only one embryo selected for transfer, respectively. The mean reduction in day-5 embryos suitable for transfer was (A) 2.55 embryos (SD: 1.87), (B) 2.31 embryos (SD: 1.83) and (C) 2.67 embryos (SD: 2.07).

The agreement for the classification of embryos as suitable for transfer was found to be good between algorithm A and B (kappa = 0.725, 95% CI 0.659–0.790). These algorithms showed no agreement with algorithm C (kappa = 0.072, 95% CI – 0.023–0.167; 0.040, 95% CI – 0.053–0.123, respectively). Fair kappa scores were seen for assessment of agreement between the embryo(s) selected by the algorithms and the embryo that was chosen by the majority (>5) of embryologists when day-5 images, as well as when day-3 and day-5 images were provided. The agreement between the embryos identified as selectable for transfer by the algorithms and the ones that were deemed selectable in the laboratory (i.e., transferred or cryopreserved) on the day of each transfer was poor or non-existent (algorithm A: – 0.007, 95% CI – 0.092–0.078; algorithm B: 0.019, 95% CI – 0.069–0.107; algorithm C: 0.068, 95% CI – 0.012–0.149).
Limitations, reasons for caution: Only three algorithms were identified for analysis, two of which (A and B) were designed by the same group of authors.
Wider implications of the findings: If the tested algorithms were indeed selecting the embryo with the highest implantation potential, then high agreement would be expected. The results of this study raise concerns as to whether these algorithms are applicable in a different setting, emphasizing the need for proper external validation before any large-scale clinical use.
Trial registration number: N/A.

O-225 A meta-analysis of the benefit using time-lapse imaging in assisted reproduction to improve pregnancy rate and reduce early pregnancy loss

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Study question: Does a meta-analysis of existing RCTs on human embryo assessment favor time-lapse imaging or conventional morphology assessment for ongoing clinical pregnancy and early pregnancy loss.
Summary answer: The results favor time-lapse compared to conventional embryo assessment for pregnancy. This is strengthened by the use of algorithms, which also reduce early pregnancy loss.
What is known already: The introduction of time-lapse imaging systems in human IVF has caused discussions about how new technologies shall be implemented in daily clinical routines. It has been suggested that the clinical benefits of applying new technologies shall be verified and documented by randomized controlled trials (RCTs) before routine implementation into clinical IVF. A Cochrane review based on three randomized trials concluded that there was insufficient evidence for the benefit of time-lapse imaging, although the authors of this study questioned this review. However, in the meanwhile three further RCTs were published, justifying another meta-analysis on this subject.
Study design, size, duration: A comprehensive search of major databases was undertaken for trials that randomized patients to time-lapse based embryo culture and assessment, with or without using an algorithm, or to conventional embryo assessment. Eligible studies were screened for number of patients treated. For ongoing pregnancy rate and early pregnancy loss absolute numbers of positive βhCG and clinical pregnancies with fetal heartbeat were evaluated. Missing data were requested from authors of the respective RCTs.
Participants/materials, setting, methods: A meta-analysis was performed on the selected studies and subsequently on the subset of studies that used an

algorithm. For the meta-analysis, the fixed or random effect model was applied when appropriate. For the statistical analysis, proportions were presented as percentages and compared using a 2 × 2 Chi-square test. The analysis were performed using MedCalc® 16.1 with *p*-values <0.05 considered statistically significant.

Main results and the role of chance: Six studies based on randomization of 1893 patients were included. The meta-analysis showed an increase of the ongoing pregnancy rate from 40.9 to 46.8% by using time-lapse for continuous embryo assessment compared to the conventional approach at fixed time points (odds ratio 1.366; 95% CI: 1.130–1.652; $p = 0.001$). Early pregnancy loss did not reach significance in these six studies ($p = 0.051$). Four of the studies applied, in addition to time-lapse imaging, an algorithm for selecting embryos for transfer and randomized 1465 patients. In these studies the results for ongoing pregnancy were further strengthened (41.9 versus 53.2%; odds ratio 1.525; 95% CI: 1.235–1.884; $p < 0.001$) and the early pregnancy loss was significantly lowered (21.0–15.2%; odds ratio 0.664; 95% CI: 0.465–0.947; $p = 0.024$) by time-lapse assessment. Repeating the evaluations by using the absolute numbers for positive/negative results and comparing with Chi-square test supported the results of the meta-analysis and showed a significant improvement in ongoing pregnancy and a significant decrease in early pregnancy loss for all study groups compared to controls.

Limitations, reasons for caution: This meta-analysis was based on the currently available RCTs. The design of these studies differs from one to another and further studies that use similar protocols may be valuable. Algorithms in the RCTs were based on clinics own data. It is not advisable using these algorithms directly.

Wider implications of the findings: This meta-analysis underscores the growing evidence for the clinical benefit of using time-lapse imaging systems in human IVF. Reduced early pregnancy loss and higher ongoing pregnancy after embryo assessment by time-lapse does result in a shorter time to pregnancy and is further strengthened by the use of an algorithm.

Trial registration number: Not applicable.

SELECTED ORAL COMMUNICATIONS

SESSION 61: NOVEL MARKERS IN REPRODUCTIVE ENDOCRINOLOGY

Wednesday 06 July 2016 **Hall 5 CB** **10:00–11:45**

O-226 Activin A production and actions during three-dimensional culture of macaque ovarian follicles – a potential noninvasive biomarker for follicle and oocyte growth *in vitro*

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Study question: What are the production patterns and direct actions of activin A during *in vitro* follicle maturation in primates?

Summary answer: While activin A production levels correlated positively with follicle and oocyte growth rates, supraphysiologic levels of activin A suppressed follicular development and function *in vitro*.

What is known already: Activin A is expressed in the adult ovary, particularly in developing follicles. Studies in rodents and domestic animals investigated activin A actions *in vitro* using cell and follicle culture, and results varied in terms of activin A effects on follicle growth and steroid hormone production.

Study design, size, duration: *In vitro* follicle maturation was performed using rhesus macaques ($n = 4$; 4–7 years old). Secondary follicles (diameter = 125–225 µm) were mechanically isolated from ovaries obtained at days 2–4 of the menstrual cycle, encapsulated into alginate (0.25% w/v), and cultured for 5 weeks with or without activin A treatment. Data were analyzed using a two-way ANOVA with repeated measures. A Student's *t* test was used to compare between two groups at each time point.

Participants/materials, setting, methods: Individual follicles (36–48 follicles/monkey) were cultured in a 5% O_2 environment, in alpha minimum essential medium supplemented with recombinant human follicle-stimulating hormone and insulin. Follicles were randomly assigned to two groups: (a) media-only control and (b) 50 ng/ml recombinant human activin A during week 3–5 (after antrum formation). Follicle survival, growth, and oocyte size were assessed. Culture media were analyzed weekly for estradiol, progesterone (immunochemiluminescence), and total activin A (control media only; ELISA).

Main results and the role of chance: In the control group, macaque follicles that survived to grow formed an antrum at week 3. The *in vitro*-developed small antral follicles (diameter = 0.5–1.5 µm) were divided into distinct cohorts based on production of activin A. While media activin A remained undetectable (<40 pg/ml) for 72% follicles throughout 5 weeks of culture, 28% follicles started to produce activin A at week 3 with media concentrations increased at week 5 (113 vs. 264 pg/ml; $P < 0.05$). Diameters of follicles and their enclosed oocytes were greater at week 5 in follicles producing activin A relative to those without activin A production (847 vs. 736 µm; 112 vs. 108 µm; $P < 0.05$). While exogenous activin A during week 3–5 did not alter follicle survival, activin A exposure decreased diameters of *in vitro*-developed small antral follicles and their enclosed oocytes at week 5 compared with controls (672 vs. 777 m; 87 vs. 109 µm; $P < 0.05$). Steroid hormone production by growing follicles increased after antrum formation. Follicles cultured with activin A during week 3–5 had lower media concentrations of estradiol and progesterone compared with controls at week 5 (2377 vs. 3324 pg/ml; 9 vs. 80 ng/ml; $P < 0.05$).

Limitations, reasons for caution: This study reports activin A production and actions during *in vitro* maturation of individual macaque follicles limited to the interval from the secondary to small antral stage.

Wider implications of the findings: Active activin A production by small antral follicles may serve as noninvasive biomarker indicating the potential of follicle and oocyte growth in primates. The findings may be applied to human *in vitro* follicle maturation, by selecting follicles for further culture or oocyte maturation, to offer fertility preservation options to women.

Trial registration number: Not applicable.

O-227 Lessons learnt from a dedicated, tertiary, multidisciplinary clinic for Mayer Rokitansky Küster Hauser Syndrome

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Study question: We present the lessons learnt from our experience of a dedicated clinic for Mayer Rokitansky Küster Hauser Syndrome (MRKH).

Summary answer**:** There is considerable variation in the approach to the management of these patients and this study highlights that referral to tertiary services is timely.

What is known already: MRKH is a condition that affects 1 in 4500 females. It is characterised by the absence of a functioning uterus, an absent or short vagina, normal secondary sexual characteristics, normal external genitalia and a normal female karyotype. The medical care of these patients in a multidisciplinary setting involves gynaecologists, nurse specialists, psychologists and radiologists.

Study design, size, duration: A retrospective analysis of 213 MRKH patients was carried out between 2010 and 2015. Out of 213 case notes, 152 cases where data was complete were selected for final analysis.

Participants/materials, setting, methods: Participants were selected from a dedicated, multidisciplinary clinic for the care and management of MRKH patients at a tertiary care centre in London, UK. A dataset was completed and analysed for age of presentation, diagnostic tests, patient desires for treatment, MRI features of the Müllerian and Wolffian duct structures, other co-existing conditions and management outcomes. Statistical analysis was carried out using SPSS.

Main results and the role of chance: The average age at presentation to secondary care was 16 years old (23.3%, IQR 16–18 years) and the average age at presentation to tertiary care was 17 years old (21%, IQR 16–19 years). Although primary amenorrhoea was present in all, 6% of patients presented with inability to have penetrative sexual intercourse and 4.6% presented with cyclical pelvic pain. In MRI imaging, rudimentary uteri were found in 90%, out of which 40.5% had bilateral uterine buds. Adenomyosis and endometriosis were seen in 11.7% and four patients had functional endometrium requiring removal of the rudimentary uteri. 16.6% of patients had examinations under anesthesia and diagnostic laparoscopies before referral. Polycystic ovary syndrome (PCOS) diagnosed by hyperandrogenism and polycystic ovaries was noted in 4.6% and two patients had premature ovarian insufficiency. 22% of patients had renal abnormalities, 3.3% had skeletal abnormalities. 63.2% of patients

embarked on a vaginal dilation programme, 34% did not require dilation and 12% deferred any treatment. 67% of patients completed vaginal dilation programme. Vecchietti procedure was performed in nine patients who did not have success with dilator therapy. Of these, four patients were unable to have sexual intercourse despite vaginas of adequate length and capacity.

Limitations, reasons for caution: We have not analysed psychological outcomes and fertility as a part of this study.

Wider implications of the findings: Rudimentary uteri are typically present and a small proportion contain functional endometrium. Some patients undergo unnecessarily invasive tests in secondary care due to diagnostic uncertainty. Failure rates in Vecchietti procedure highlight that women who are unsuccessful with dilator therapy may not be successful due to non-compliance with dilation after surgery.

Trial registration number: N/A.

O-228 Gonadotrophin secretion is a useful adjunct in the assessment of patients with hyperprolactinaemia

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Study question: Can evaluation of gonadotrophin secretion assist in the diagnosis of patients with hyperprolactinaemia?

Summary answer: Gonadotrophin secretion aids the diagnosis of patients with hyperprolactinaemia. Patients with PCOS have LH-predominant secretion, whereas patients with Microprolactinomas/Drug-Induced Hyperprolactinaemia have FSH-predominant secretion.

What is known already: Hyperprolactinaemia accounts for one in seven patients presenting with amenorrhoea and infertility.

Recent data suggests that prolactin acts at the level of kisspeptin-neurons in the hypothalamus to reduce GnRH-pulsatility.

Conditions in which GnRH-pulsatility is reduced, favour FSH-predominant secretion from the pituitary gland. However conditions in which GnRH-pulsatility is increased, such as PCOS, favour LH-predominant secretion.

Thus, causes of hyperprolactinaemia such as Drug-Induced Hyperprolactinaemia (DIH) or Microprolactinoma, would be expected to have reduced GnRH-pulsatility and FSH-predominant gonadotrophin secretion.

Patients with hyperprolactinaemic conditions in which pituitary gonadotroph function is directly impaired, such as Macroprolactinoma, would be expected to have attenuated gonadotrophin secretion.

Study design, size, duration: A retrospective analysis of gonadotrophin secretion in patients presenting with hyperprolactinaemia during June 2012–October 2015 was performed. 302 patient records were reviewed and 262 patients with monomeric hyperprolactinaemia and gonadotrophin evaluation were identified (166 female, 96 male). The most frequently encountered diagnoses were Microprolactinoma (n = 86), Macroprolactinoma (n = 45), Non-Functioning Microadenoma (NFMI) (n = 15), Non-Functioning Macroadenoma (NFMA) (n = 90), Drug-Induced Hyperprolactinaemia (DIH) (n = 12) and Polycystic Ovarian Syndrome (PCOS) (n = 14).

Participants/materials, setting, methods: Concomitant serum FSH and LH levels in patients with a raised serum monomeric prolactin level over the gender-specific reference range were recorded at Imperial College Healthcare NHS Trust, London, UK.

The cause of hyperprolactinaemia was diagnosed by an experienced reproductive endocrinologist at a tertiary centre, unless pituitary surgery yielded a histologically confirmed diagnosis. Groups were compared by one-way ANOVA with post hoc Tukey's. Mean gonadotrophin values are presented. P-value 0.05 was regarded as statistically significant.

Main results and the role of chance: Gonadotrophin secretion in Drug-Induced Hyperprolactinaemia (DIH) (FSH 4.5 IU/L, LH 3.1 IU/L, FSH-LH +1.4 IU/L) was similar to that observed in Microprolactinomas (FSH 4.6 IU/L, LH 3.8 IU/L, FSH-LH +0.8 IU/L). As expected, patients with DIH or Microprolactinoma have FSH-predominant gonadotrophin secretion consistent with reduced GnRH pulsatility, due to the action of prolactin at the level of the hypothalamus.

However, in patients with PCOS and hyperprolactinaemia, LH-predominant secretion was observed, consistent with increased GnRH pulsatility (FSH 3.5 IU/L, LH 5.1 IU/L, FSH-LH −1.6 IU/L).

In patients with Macroprolactinoma, serum gonadotrophin secretion was reduced when compared with Microprolactinoma consistent with direct pituitary gonadotroph infiltration and hypo-function in patients with Macroprolactinoma (FSH 3.0 IU/L, LH 2.4 IU/L, FSH-LH +0.5 IU/L) (serum LH $P < 0.05$ vs. Microprolactinoma).

In patients with Macroadenomas, those with "Non-Functioning Macroadenomas" (NFMA) had greater FSH secretion than those with Macroprolactinoma (NFMA: FSH 5.9 IU/L, LH 2.4 IU/L, FSH-LH +3.8 IU/L (FSH $P <$ 0.05 vs. Macroprolactinoma; FSH-LH $P < 0.05$ vs. Macroprolactinoma). This suggests that whilst nominally "Non-Functioning," many NFMA's secrete FSH at subclinical levels. This observation may assist in the assessment of patients with macroadenoma, differentiating patients with Macroprolactinoma from those with NFMA and disconnection hyperprolactinaemia (Area Under Curve of ROC analysis for FSH-LH is 0.67, 95% CI 0.57–0.76).

Limitations, reasons for caution: Causes of hyperprolactinaemia were diagnosed by experienced clinicians in reproductive endocrinology at a tertiary centre, however not all patients required pituitary surgery and thus not all diagnoses were histologically confirmed.

Wider implications of the findings: Reproductive specialists may use these data in the diagnosis of patients with hyperprolactinaemia. These data may assist physicians in the identification of patients with PCOS with mildly elevated prolactin levels. Patients on drugs causing hyperprolactinaemia, but with reduced gonadotrophin secretion, may require urgent assessment to exclude causes of hypopituitarism.

Trial registration number: This retrospective analysis was not a registered clinical trial.

O-229 TOP001, a novel orally active allosteric agonist of follicle stimulating hormone (FSH) receptor, stimulates similar maturation of high quality oocytes in preclinical models as hFSH

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Study question: Can orally administered TOP001 stimulate increases in follicular growth, plasma estradiol, oocyte fertilization and blastocyst development in animal models comparable to injected recombinant hFSH (rec-hFSH)?

Summary answer: TOP001 and rec-hFSH stimulated similar dose-dependent increases in follicular growth and estradiol production in rats, and blastocyst development rates in mice.

What is known already: Last year, we presented the development of orally active FSHR allosteric agonists through a rational drug discovery effort. TOP001, the lead compound, stimulated cAMP in CHO cells expressing hFSHR with an EC50 of 9.8 nM, while it had 6 & 50 fold less activity in hLHR and hTSHR expressed cells, respectively. TOP001 dose dependently stimulated estradiol production in rat and human granulosa cell cultures, and it has been shown to stimulate follicular development *in vivo*. TOP001 has been shown to have remarkable safety profile in *in vitro* safety and selectivity assays, and in *in vivo* toxicology assessments in rats and dogs.

Study design, size, duration: Immature rats were treated for 2 days with low (0.29 IU) or high dose FSH (1.16 IU), while TOP001 was administered orally (1–100 mg/kg) to evaluate follicular growth either in presence or absence of FSH. Serum estradiol was measured to monitor follicular growth. Immature mice received TOP001, rec-hFSH or PMSG, and oocytes were isolated, fertilized, and embryo development to blastocysts was measured. Each experiment was repeated at least twice with n = 5 animals/group.

Participants/materials, setting, methods: This study was performed in a preclinical drug discovery setting. Animals were maintained and treated with injectable FSH, PMSG or orally active TOP001 according to approved animal care protocols.

Main results and the role of chance: In immature rats, oral administration of TOP001 at 5, 10 & 25 mg/kg along with injection of low dose FSH (0.29 IU), significantly increased the number of oocytes (22.1 + 1.9; 50.5 + 3.4 & 56.8 + 3.4 respectively, $p < 0.05$; ANOVA) compared to low dose FSH alone (6.8 + 0.9). Significant increases in serum estradiol were observed at the doses of 10 & 25 mg/kg (94.5 + 13.2; 94 + 9.6 pg/ml respectively) over low FSH alone (35 + 7.3 pg/ml). Similar increases in oocyte and serum estradiol were observed when animals were treated orally with TOP001 alone. Maximal serum estradiol (87.3 + 10.2 pg/ml) and number of oocytes in oviduct (42.5 + 3.3) were similar for rec-hFSH and TOP001. The ovarian stimulation

protocol was adopted to immature mice with similar increases in follicular growth for TOP001 (1–100 mg/kg), rec-hFSH and PMSG. The percent of MI & MII oocytes retrieved following oral administration TOP001 at 30 mg/kg (68.6 & 31.4) & 100 mg/kg (84.8 & 15.2) was similar to the high dose rec-hFSH (71.3 & 28.7). The fertilization rate of oocytes from TOP001 treated animals (30 mg/kg–76.5 + 8.9%; 100 mg/kg–77 + 6.2%) was not different from those obtained from rec-hFSH (73.2 + 6.1%) or PMSG treated groups (87.2 + 5.7%). Furthermore, development of blastocysts among TOP001, rec-hFSH and PMSG groups were similar.

Limitations, reasons for caution: The compound is in early evaluation of safety and toxicology prior to initiating GLP studies for IND filing. The safety and efficacy of the compound in humans will be determined in 2017.

Wider implications of the findings: TOP001 shows promise to be a novel and convenient therapy for controlled ovarian stimulation, as demonstrated by significant increases in follicular growth, and plasma estradiol. Similarly, TOP001-derived oocytes is shown to lead to comparable blastocyst development rates as rec-hFSH and PMSG, reflecting the overall safety of this compound on oocytes.

Trial registration number: Only preclinical animal models have been used in this research.

O-230 Cell-free DNA level in serum, as a new biomarker for prognostic prediction of ovarian response to stimulation

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[4]*Clinique Pierre Cherest, IVF Department, Neuilly-sur-seine, France*

Study question: Could cell-free DNA (cfDNA) level in serum at day 3 of menstrual cycle be clinically useful for prediction of patient's ovarian response to stimulation during *in vitro* fertilization (IVF) procedure?

Summary answer: Cell-free DNA levels in serum at day 3 of menstrual cycle can predict significantly the number of retrieved oocyte, independently of Anti-Müllerian hormone (AMH) concentrations.

What is known already: DNA fragments, detected in blood and in other biological fluids, are released from apoptotic and/or necrotic events. Cell-free DNA level in serum is increased in some cancers or gynecological and obstetrics disorders and currently used as a non-invasive biomarker for their early detection and/or prognosis. Sometimes, AMH concentration, classically measured in patient's serum at day 3 of menstrual cycle can be inconsistent to predict ovarian response to stimulation. The potential cfDNA level in serum for the prediction of ovarian response remains to be investigated in order to identify a supplemental prognostic tool for improving the management of ovarian stimulation treatment.

Study design, size, duration: This prospective study included 32 serum samples collected at day 3 of menstrual cycle from patients undergoing IVF/ICSI procedure. AMH and cfDNA levels were measured in each serum sample in order to compare their predictive value for patient's ovarian response to stimulation.

Participants/materials, setting, methods: Serum samples were prepared in Proteinase K buffer and the total cfDNA was quantified by qPCR, using ALU 115 primers. CfDNA concentration was determined based on a standard curve obtained by successive dilution of genomic DNA. The *p*-values were calculated by using the unpaired *t*-test, Spearman correlation, multiple and logistic regressions and ROC curve analysis on GraphPad and Medcalc softwares.

Main results and the role of chance: Cell-free DNA concentrations (mean ± SD = 152.02 ± 160.48 ng/m, median = 92 ng/ml) were significantly and positively correlated with patient's age ($r = 0.18$; $p = 0.02$). Indeed, cfDNA levels were significantly higher in serum from older women (≥38 years) than those from young patients (<38 years) ($p = 0.035$). Very interestingly, cell-free DNA level was significantly related to the number of oocyte collected at oocyte retrieval ($p = 0.031$). Furthermore, cfDNA levels predicted significantly a low ovarian response (<6 retrieved oocytes), independently of AMH concentrations [Adjusted Odd Ratio = 1.01 (1.0–1.02); $p = 0.02$]. The area under the ROC curve (AUC), which quantifies the low ovarian response prediction of cfDNA level was 0.79 (0.61–0.91) with higher sensitivity (73.33%) and specificity (82.35%) ($p = 0.001$) than AMH level [AUC = 0.72 (0.54–0.87); sensitivity = 60%; specificity = 88.2%, $p = 0.02$].

Limitations, reasons for caution: Further investigations with large number of patients and their attempt outcomes will be conducted to confirm the clinical utility of cfDNA for ovarian response prediction.

Wider implications of the findings: High cfDNA levels in serum could be significantly related to a low ovarian response. Cell-free DNA quantification at day 3 could be used as a supplemental tool to identify more accurately women with a risk of poor ovarian response, in order to develop a personalized care program for these patients.

Trial registration number: N/A.

O-231 New insight into the pathogenesis of ovarian hyperstimulation syndrome (OHSS): pigment epithelium-derived factor (PEDF) targets both angiogenic and inflammatory pathways

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Study question: Could pigment epithelium-derived factor (PEDF) function as a physiological negative modulator of both angiogenic and inflammatory pathways in ovarian hyperstimulation syndrome (OHSS)?

Summary answer: Recombinant PEDF (rPEDF) possesses potent therapeutic properties in OHSS, manifested by independent dual negative regulation of the angiogenic and inflammatory pathways, which govern OHSS.

What is known already: OHSS is a potentially life-threatening complication of ART, induced by an ovarian release of vasoactive, angiogenic substances; resulting in vascular leakage. The consequent ascites is attributed to hCG-induced VEGF increase, as well as to lysophosphatidic acid (LPA)-induced increase of angiogenic cytokines interleukin (IL)-6 and IL-8.

Recently, we have shown that PEDF, a potent anti-angiogenic factor that counteracts VEGF, plays a fundamental role in the pathogenesis and treatment of OHSS. Our aim was to elucidate the putative role of the anti-angiogenic and anti-inflammatory mediator, PEDF, as a physiological negative regulator of both VEGF and angiogenic cytokines networks.

Study design, size, duration: OHSS was induced in 5-weeks old ICR female mice by three consecutive daily injections of 20 IU PMSG, followed 24 h later by 7 IU hCG. Control mice were given a single dose of 5 IU PMSG, followed 48 h later by 7 IU hCG (six mice per experimental group). To test PEDF therapeutic abilities we injected rPEDF together with hCG and recorded changes in body weight and in peritoneal vascular leakage, as quantified by the modified Miles vascular permeability assay.

Participants/materials, setting, methods: We used a mouse OHSS model and cultured granulosa cells (primary – human; cell line – rat). Changes in the levels of PEDF, VEGF and IL-6/IL-8 were measured by qPCR, western blot and enzyme-linked immunosorbent (ELISA). OHSS symptoms were recorded by changes in body weight, ovarian weight and by peritoneal vascular leakage.

Main results and the role of chance: We found that stimulation by LPA resulted in a significant increase of IL-6/8 mRNA and protein levels in granulosa cells, inversely to PEDF level ($P < 0.01$). In addition, we found that OHSS was correlated with hCG-induced impaired PEDF/VEGF ratio. Interestingly, triggering with GnRH-agonist, which is known to prevent OHSS, modulated PEDF/VEGF ratio inversely to that induced by hCG-triggering, both *in vitro* and *in vivo*. Stimulation with rPEDF reduced the LPA-induced increase of IL-6\8 levels in human granulosa cells ($P < 0.01$). Moreover, *in vivo* treatment with rPEDF (0.5 mg/kg) alleviated OHSS signs including edema ($P < 0.05$), vascular leakage ($P < 0.01$) and reduced ovarian IL-6 mRNA and protein levels ($P < 0.01$).

Limitations, reasons for caution: The *in vivo* experiments were performed in a mouse model.

Wider implications of the **findings:** Our findings provide new perspectives into the role of PEDF in OHSS pathophysiology: low level of PEDF expression enables high levels of VEGF and IL-6/8 expression. Exploring the anti-angiogenic and anti-inflammatory properties of PEDF in the female reproductive system could open new therapeutic avenues for other fertility and gynecological pathologies.

Trial registration number: Not relevant.

O-232 Vitamin D deficiency and pregnancy rates following frozen-thawed embryo transfer: a prospective cohort study

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Study question: What is the effect of vitamin D deficiency on the pregnancy rates in frozen-thawed embryo transfer cycles?
Summary answer: Vitamin D deficiency does not affect pregnancy rates in frozen-thawed embryo transfer cycles.
What is known already: Although there is evidence that the potential impact of vitamin D deficiency on reproductive outcome may be mediated through a detrimental effect on oocyte or embryo quality, the rationale of our design was based on evidence derived from basic science suggesting that vitamin D may have a key role in endometrial receptivity and implantation. Only few retrospective clinical studies have been published up to date with conflicting results.
Study design, size, duration: This study is a prospective cohort study from the Centre for Reproductive Medicine at the University Hospital of Brussels. The duration of the study was 1 year.
Participants/materials, setting, methods: A total of 280 consecutive patients who had at least 1 blastocyst frozen and were planned for a frozen-thawed embryo transfer, were enrolled in the study following detailed information and signing of a written informed consent. Serum analysis of 25-OH vitamin D was measured on the day of embryo transfer and the impact of vitamin deficiency was investigated on reproductive outcomes.
Main results and the role of chance: Among all patients, 45.3% ($n = 127$) had vitamin D deficiency (<20 ng/ml), and 54.6% ($n = 153$) had normal vitamin D levels (>20 ng/ml). Positive hCG rates were similar among patients with vitamin D deficiency and women with normal total serum 25-OH vitamin D levels (40.9 versus 48.3%, $p = 0.2$). Similarly, no difference was found in clinical pregnancy rates in women with vitamin D deficiency [32.2% (41/127)] compared to those with higher vitamin D levels [37.9% (58/153)]; $p = 0.3$. When analyzing the results according to different thresholds, as proposed by the Endocrine Society, clinical pregnancy rates were comparable between vitamin D deficient (<20 ng/ml), vitamin D insufficient (20–30 ng/ml) and vitamin D replete women (≥30 ng/ml) [32.3% (41/127) versus 39.5% (36/91) versus 35.5% (22/62), respectively, $p = 0.54$]. Multivariate logistic regression analysis showed that vitamin D status is not an independent predictive factor of clinical pregnancy.
Limitations, reasons for caution: Ethnicity was not assessed, given that the vast majority of patients were Caucasian. Furthermore, although we failed to find a difference between vitamin D deficient and non-deficient women, we need to underscore that our study was powered to detect a difference of 15% in clinical pregnancy rates.
Wider implications of the findings: Vitamin D deficiency does not significantly impair pregnancy rates among infertile women undergoing frozen-thawed embryo transfer cycles. The measurement of vitamin D levels in this population should not be routinely recommended.
Trial registration number: The trial was prospectively registered in clinicaltrials.gov as NCT01985672.

SELECTED ORAL COMMUNICATIONS

SESSION 62: OOCYTE AND EMBRYO CRYOPRESERVATION

Wednesday 06 July 2016 **Hall 5 A** **10:00–11:45**

O-233 A new device for warming embryo during the transfer improves clinical outcomes. Preliminary results in a RCT

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Study question: To determine whether protecting embryos from thermal stress with the use of a new device can improve clinical outcomes.
Summary answer: By maintaining the temperature at 37°C during the embryo transfer procedure, we increase success rates in IVF.
What is known already: The human embryo is very sensitive to adverse conditions. *In vivo* fertilization and cleavage preserve it from changes in the temperature. During the embryo transfer procedure, the embryo is transported from the incubator to the maternal uterus inside a soft catheter. Despite the lab temperature being around 24°C, we have determined a temperature drop in the catheter, in which it falls from 37 to 29°C in just 40 s. Keeping embryos in these suboptimal conditions could negatively affect their development.
Study design, size, duration: After the Ethics Committee approval, we carried out a randomized control trial study. Patients were assigned to group A (control: conventional embryo transfer procedure) or group B (study: catheter carried in the device). The objective is to observe if a 15% increase in success rate is achieved in group B. The number of data collected must be 176 cases in each group. The period of time has not been established. The results were analyzed periodically.
Participants/materials, setting, methods: Only cryotransfers of embryos from donated oocytes were included. Exclusion criteria are: patients suffering from repeat implantation failure, recurrent pregnancy losses or anatomic abnormalities in uterine cavity.

After being informed and signing a consent form, patients were randomly assigned to group A (control group) or group B (study group).
Main results and the role of chance: Before launching this study, we confirmed the accuracy of maintaining the temperature in this new device. We warmed up the device inside the incubator at 37°C overnight. We then tested the temperature drop inside the device at room temperature during a long period of time. We observed a slow temperature fall, registering 36.5°C inside the device 10 min after exposure to room temperature.

We analyzed our preliminary data with a χ-test to evaluate differences. 66 treatments were included [27 control group (A), 39 study group (B)]. An improvement in positive pregnancy tests in the study group was observed, 44.4 vs. 51.3% ($p = 0.624$). No statistical significance was obtained with high probability due to the low number of cases, considering that we need 176 treatments in each group. Despite the low number of reported cases, from the very beginning there was a trend of higher success rates in transfers using the thermal device.
Limitations, reasons for caution: These are preliminary results and we need a higher number of cases to confirm our hypothesis. However, we hope to confirm them briefly since the results are encouraging.
Wider implications of the findings: Taking care of the embryo during the whole IVF procedure including the embryo transfer should be mandatory. Maintaining the optimal temperature until the embryo arrives in the maternal uterus using this new device could improve the success rates.
Trial registration number: NCT02650310.

O-234 Hydroxypropyl cellulose is an effective surfactant agent for vitrification solutions: a prospective study on donor oocytes

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Study question: Test the effectiveness of a protein-free vitrification solution supplemented with the synthetic macromolecule hydroxypropyl cellulose (HPC) as surfactant agent for the vitrification of human donor oocytes.
Summary answer: Vitrification of human donor oocytes with a protein-free vitrification solution yields excellent laboratory outcomes, comparable to fresh sibling oocytes.
What is known already: Protein supplementation with human serum albumin has traditionally been employed as surfactant agent for the vitrification and rewarming solutions. However, it presents risks of contamination and production variability so a replacement synthetic component has been sought for long. A fully synthetic formulation is desirable: the international regulatory policies are also encouraging the use of medias with these synthetic substitutes. The synthetic macromolecule HPC is a variable length polysaccharide that forms a viscous gel under low temperatures. HPC based vitrification solutions have shown promissory results on murine and human embryos.
Study design, size, duration: A prospective study including 219 donor MII oocytes. Depending on the availability, each recipient was assigned 6–9 fresh

MII oocytes (control group) and 2–5 vitrified/re-warmed MII oocytes (experimental group) from the same donor of a previous donation cycle. Both groups of oocytes underwent ICSI and were cultured in parallel. Primary performance end-point was the fertilization rate. Secondary parameters assessed were embryo quality in day 2 and day 3.

Participants/materials, setting, methods: Oocytes underwent a 8–12 min equilibration step (ES: 7.5% ethylene glycol EG, 7.5% DMSO), rinsed in vitrification solution for 60 s (VS: 15% EG, 15% DMSO), and loaded in a kitazato open carrier (Kitazato, Japan). Occytes were warmed in 1.5 mL of thawing solution for 60 s (TS, 1M sucrose), placed for 3 min in dilution solution (DS: 0.5M sucrose) 5 min in washing solution (WS: cryoprotectant free solution). Vitrification solutions by SafePreservation, Spain.

Main results and the role of chance: Out of 73 vitrified MII oocytes, 70 (95.9%) presented morphologic survival 2 h post warming, and 49 of them (70.0%) presented normal fertilization, compared to 105 (71.9%) of 141 MII fresh oocytes ($p > 0.05$) (Table 2). In day 2, 82.2% of embryos from vitrified oocytes were of good quality, against a 71.7% from fresh oocytes, and 69.2% against a 70.0% of good quality embryos on day 3, respectively ($p > 0.05$). 15 embryos were transferred in total from both fresh and vitrified oocytes, with an implantation rate of 53.3%.

Limitations, reasons for caution: Only the extended use of HPC based vitrification solutions over time and in a wide variety of patients will provide good quality evidence of these findings.

Wider implications **of the findings:** The satisfactory results of this direct comparison with fresh controls is a strong indicator of the safety and effectivity of HPC supplementation of vitrification solutions, confirming its role as a substitute of protein supplementation.

Trial registration number: N/A.

O-235 Oxidative markers in cryopreservation medium of frozen- thawed embryos in *In Vitro* Fertilization: a possible tool for improved embryo selection?

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Study question: Is there an association between oxidative parameters in embryo cryopreservation medium measured by the Thermochemiluminescence (TCL) assay and laboratory and clinical outcome parameters?

Summary answer: Thawed embryos may express oxidative processes in the cryopreservation medium. Higher oxidative levels are associated with lower implantation rates.

What is known already: Cryopreservation of surplus embryos has been routine practice in IVF laboratories. Both cryopreservation and thawing have been associated with post-thaw damage *via* several mechanisms, including oxidative stress. Embryos chosen for transfer following a freeze-thaw cycle are assessed on the basis of a subjective morphological grading and scoring system. The TCL assay has been used to investigate oxidative stress in biological fluids and been validated in several studies using various biological samples. In a previous study, we found that TCL oxidative parameters in the embryo culture media may serve as quick, simple, and accurate tools for embryo selection in IVF.

Study design, size, duration: A Prospective, cohort clinical and laboratory study. Study population included ninety one IVF patients undergoing frozen-thawed embryo transfer cycle during 2013.

Participants/materials, setting, methods: The Study was held in an IVF unit in a university-affiliated hospital were included. Following thawing, 50 microliters of embryo cryopreservation medium were retrieved from each cryotube and tested in the TCL Analyzer. Recorded parameters were: TCL amplitudes after 50 (H1), 150 (H2) and 280 s (H3) in counts per seconds (CPS) and TCL ratio (%). These were compared with clinical and laboratory parameters including implantation and pregnancy rates. Data underwent statistical analysis.

Main results and the role of chance: Altogether 194 embryos were transferred in 85 frozen-thaw cycles. Twenty-one pregnancies (24.7%) occurred. In the ROC analysis, the median TCL H1 amplitude of 32 CPS was the best cut-off value for discriminating between conception and non-conception cycles. Implantation, overall and clinical pregnancy rates were higher when TCL H1 amplitude was <32 CPS (median) compared with TCL H1 ≥ 32 CPS (14.6 vs. 5.3, 37.5 vs. 17, 28.1 vs. 9.4%, respectively). In addition, higher pregnancy rates were associated with the transfer of embryos at 2 days, a greater number of transferred embryos, and the transfer of embryos with a lower TCL H1 amplitude, or a TCL H2 amplitude <35 CPS. No pregnancies occurred when H1 amplitude was ≥40 CPS. Logistic regression multivariate analysis found that only TCL H1 median amplitude was associated with the occurrence of pregnancy (OR = 2.93, 95% CI = 1.065–8.08). TCL ratio inversely correlated the duration of embryo cryopreservation ($r = -0.37$). The median TCL H3 amplitude was higher among smokers than non-smokers.

Limitations, reasons for caution: As a TCL H1 amplitude >40 CPS was found in only five cases, it is premature to recommend that embryos be discarded from transfer if this specific value is measured. This small- scale preliminary study warrants a larger, prospective, randomized, controlled study, before clinical recommendations may be made.

Wider implications of the findings: Results warrant a large-scale prospective study using cryopreservation medium from vitrified embryos, (cleavage stage and single blastocysts). As cryopreservation of embryos becomes more common, we believe that application of the TCL assay may provide an additional quick, non-invasive, simple, accurate, and objective tool for better embryo selection after thawing.

Trial registration number: Since this study was not an interventional study, it was not registered.

O-236 Different thawed cleaved embryo transfer strategy for cryopreserved embryos according to mode of cryopreservation?: a prospective randomized study comparing 2 culture medium duration

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Study question: Impact of post-thaw embryo culture duration on implantation and pregnancy rates according to the freezing techniques: vitrification vs. slow freezing.

Summary answer: While the time of embryo-culture post thawing shows no impact after slow freezing, it seems to be decisive on implantation and pregnancy rates after vitrification.

What is known already: The two most common procedures to select cleaved embryos for frozen embryo transfer (FET) are the observation of blastomere survival and proliferation after thawing, requiring a longer culture (overnight) (El-Toukhy et al., 2003). A study pointed out a potential harmful effect of the *in vitro* embryo culture of slowly frozen embryos after thawing, because of their sensibility to sub-optimal environmental conditions (Rato et al., 2012). The vitrification induces fewer traumas to cells and is, therefore, most efficient (Loutradi et al., 2008), but it requires an increase of cryoprotectant concentration.

Study design, size, duration: Prospective cohort study at Bichat-Claude Bernard Hospital (AP-HP, Paris) including 701 frozen thawed embryo cycles between March 2013 and December 2015. Day 2 embryos with 3 <6 blastomeres and ≤30% fragmentation were cryopreserved using slow controlled freezing [Embryo Freezing Pack, Origio, France] or closed vitrification [Vit KitR – Freeze, Irvine Scientific, France]. Only embryos with ≥50% of cells intact after thawing were transferred either after 2 h (group A) or overnight of culture (group B).

Participants/materials, setting, methods: A total of 449 thawed day 2 cleaved embryo cycles were included. 88 and 116 cycles after slow freezing and vitrification respectively were included in group A 125 and 120 cycles after slow freezing and vitrification respectively were included in group B Chi 2 tests were performed to assess survival, clinical pregnancy (CPR) and implantation rates (IR) according to the group and the mode of cryopreservation.

Main results and the role of chance: The number of transferred embryos was 316 and 356 after slow freezing and vitrification respectively. The survival rate was 79.9 and 85.5% after slow freezing and vitrification respectively. No significant difference was evidenced between groups A and B in terms of CPR and IR in case of slow frozen embryos (20.5 vs. 20.0, and 15.7 vs. 14.3%). However, C PR and IR were significantly higher in the group B (overnight culture) than in group A when frozen embryos were vitrified (28.3 vs. 16.4 and 22.0 vs. 11.5% respectively; $P < 0.01$).

Interestingly, to confirm the importance of culture duration on vitrified embryo outcomes, we compared results of 184 day 3 frozen embryos (132 cycles) where thawing and transfer occurred the same day. Despite a significant higher

survival rate after vitrification than slow freezing (89.3 vs. 59.0% $P < 0.01$), PR and IR were significantly better after slow freezing (27.3 and 21.2%) than after vitrification (20.0 and 17.0%, $P < 0.05$), suggesting the potential role of culture duration on thawed vitrified embryos.

Vitrified embryos need longer time of *in vitro* culture before transfer, probably to reject the main quantity of cryoprotectants.

Limitations, reasons for caution: Need to compare other stages of frozen embryo (day 1, day 5 and 6) to confirm the effect of time culture duration after vitrification.

Wider implications of the findings: With all the enthusiasm that has occurred in recent years around vitrification, mainly of blastocyst, and earnings on cell preservation, this study suggests that the effect of cryoprotectants at high concentration could be harmful, and new recommendations on frozen cleaved embryo transfer strategies could be depicted.

Trial registration number: Not applied.

O-237 Morphology dynamics of warmed blastocyst are strong predictors of clinical outcome

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Study question: Can time-lapse imaging be used to define morphology dynamics of frozen blastocysts and become indicators of embryo outcome?

Summary answer: The analysis of warmed blastocysts by cinematography is providing new insights on embryo outcome and defines new markers for blastocyst implantation potential.

What is known already: Blastocyst re-expansion has shown to have predictive properties for the implantation of warmed blastocysts. However, no quantitative values have been defined to be used as a predictor of viability.

Study design, size, duration: A retrospective study including 414 frozen embryo transfer cycles from November 2014 to December 2015.

Participants/materials, setting, methods: The study includes 435 thawed blastocysts with known implantation data, evaluated using time lapse. Blastocysts were placed in Embryoscope® (Unisense-FertiliTech) from immediately after warming until transfer (>3.5 h). Embryos were vitrified and warmed with Cryotop method (Kitazato Biopharma). Variables studied included initial and final thickness of zona pellucida (ZP) (µm), initial and final area (µm^2), area of inner cell mass (µm^2) and presence of collapse after warming procedure. Variables analyzed were compared by ANOVA test or chi-square.

Main results and the role of chance: Implantation rate (IR) was increased (33.6 vs. 20.2%) in blastocysts with initial ZP thickness <18 µm. Blastocysts with initial area >9900 µm^2 obtained higher implantation rate (33.3 vs. 22.5%). IR was higher (35.7 vs. 24.4%) when the final ZP were <12 µm and it was also significantly increased (41 vs. 26%) when the final blastocyst area reached more than 19136 µm^2. No differences were found in area of inner cell mass between blastocysts which implanted and those which failed. In 32 cases, one or more contractions of the blastocyst were observed but no relation with implantation was confirmed.

Limitations, reasons for caution: The retrospective nature of this study may be a limitation, although the magnitude of the sample size would overcome it. In addition, the time period in which embryos were analyzed may vary depending on transfer schedule.

Wider implications of the findings: These results show that the analysis of warmed blastocysts by time-lapse imaging provides new markers for implantation, establishing quantitative values linked with clinical outcome. In those cases in which the warmed blastocyst does not reach the optimal ZP thickness and full area, a double embryo transfer strategy must be considered.

Trial registration number: None.

O-238 Higher cumulative ongoing pregnancy rate after IVF cycles including a fresh embryo transfer than after freeze-all cycles

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Study question: Is there a benefit in term of ongoing pregnancy rate to perform fresh embryo transfers in IVF cycles compared to "freeze all" strategy?

Summary answer: The cumulative ongoing pregnancy rate is significantly higher in IVF cycles including a fresh embryo transfer compared to "freeze all" cycles.

What is known already: Currently, there is a trend toward the elective freezing of all embryos and subsequent replacements, so-called the "freeze all" policy. If this option is not debatable to prevent the risk of ovarian hyperstimulation syndrome (patient risk-based approach), the universal approach (cryopreservation of all embryos for all cycles) still needs to be proven.

Study design, size, duration: This cohort study includes all IVF and ICSI attempts carried out between December 2012 and April 2015 and giving rise to a pregnancy or for which all embryos were used. Women for whom the "freeze all" strategy was used (unexposed) were matched to women exposed to a first fresh embryo transfer using the propensity score, in order to reduce the recruitment bias of observational studies.

Participants/materials, setting, methods: For women who had more than one IVF attempt during the study period, only the most recent was considered. The propensity score was based on 16 individual characteristics. The main outcome was the occurrence of an ongoing pregnancy. The odds ratio (OR) was computed to quantify the association between the fresh embryo strategy and the main outcome using a logistic regression fitted by generalized estimating equations to account for paired data.

Main results and the role of chance: A total of 1624 women were included: 1068 in the fresh embryo transfer exposed group and 556 in the "freeze all" group. The propensity score matching strategy led to 398 matched exposed and unexposed women. The mean number of transfers performed in each group was 1.36 ± 0.72 and 1.29 ± 0.67 ($p = 0.139$), respectively. The cumulative ongoing pregnancy rate was 57.5% in the fresh embryo transfer exposed group vs. 40.0% in the "freeze-all" group ($p = 0.001$, OR = 2.04, 95% confidence interval: 1.54–2.69). The mean number of transfers necessary to achieved a pregnancy was 1.33 ± 0.62 and 1.37 ± 0.78 ($p = 0.581$), respectively.

Limitations, reasons for caution: Propensity score matching process could have decreased the generalizability of the results but it is a powerful tool to reduce recruitment bias. The sample size did not allow analysis of subgroups with enough power (risk of hyperstimulation syndrome, 2 prior implantation failures, premature luteinization, hydrosalpinx, endometrial polyp, endometriosis).

Wider implications of the findings: Performing a fresh transfer in the management of an IVF treatment keeps a beneficial effect on ongoing pregnancy rate compared to the freezing of all embryos, without maximizing the time to pregnancy. This emphasizes that the universal "freeze-all" approach still needs prospective randomized clinical trials to be evidence based.

Trial registration number: None.

O-239 Multi-center study demonstrates freeze-all IVF protocols are correlated with higher ongoing pregnancy rates in women of advanced maternal age.

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Study question: Questions remain about whether utilization of freeze-all protocols would improve IVF outcomes for the majority of patients.
Summary answer: Freeze-all protocols were found to result in equivalent ongoing pregnancy rates in younger patients and significantly improved outcomes in patients over 35 years old.
What is known already: As IVF with frozen embryo transfer has become more common, protocols in which all embryos are frozen and transferred in a later cycle have emerged to reduce the risk for OHSS and address concerns about endometrial receptivity (ER) in women undergoing controlled ovarian stimulation and/or experiencing a premature elevation of progesterone (P). To date, randomized control studies comparing freeze-all to fresh protocols have focused on good prognosis patients, leaving open questions about which patients might benefit most from a freeze-all protocol.
Study design, size, duration: We performed a retrospective cohort study on patients at 12 fertility treatment centers in the United States who had undergone IVF cycles in 2009–2015. We included cycles in which fresh embryos were transferred (Fresh) and cycles in which all embryos were frozen, followed by a frozen embryo transfer (Freeze-all). We excluded frozen transfers of supernumerary embryos, cancelled cycles, and cycles for which patient age, height, weight, or progesterone level at surge (hCG administration) were missing.
Participants/materials, setting, methods: 16,206 cycles were available for analysis, including 2,100 Freeze-all cycles. A matching algorithm was used to select 2,100 fresh cycles with similar distributions of measured covariates (age, diagnoses, embryos transferred, use of pre-implantation genetic screening (PGS), P at surge, bAFC, Day 3 LH, Day 3 FSH, Day 3 E2, parity, gravidity, BMI) to the Freeze-all cycles. Logistic regression was used to compare the odds of ongoing pregnancy in Freeze-all compared to matched Fresh control cycles.
Main results and the role of chance: In a matched cohort, ongoing pregnancy rates were 40.3% for Fresh and 50.2% for Freeze-all cycles. We controlled for hormone levels, number of embryos transferred, diagnosis, age, and PGS utilization and found that ongoing pregnancy rates were significantly higher in Freeze-all cycles for patients between age 35–38 [OR = 1.48 (1.14, 1.92), $p = 0.006$], 38–41 [OR = 2.59 (1.90, 3.52), $p < 0.001$], and older than 41 [OR = 8.16 (4.89, 13.59), $p < 0.001$] compared to Fresh controls. Cycles for patients younger than 35 did not have significantly different odds of ongoing pregnancy in Freeze-all compared to Fresh [OR = 1.02, (0.86, 1.22), $p = 0.83$]. Additionally, we found that a diagnosis of DOR, PCOS, endometriosis, tubal disease, uterine factor, ovulatory dysfunction, or idiopathic infertility had no significant impact on the effect of Freeze-all (all $p > 0.05$). We also observed the effect of Freeze-all versus Fresh in cycles where P at surge was greater than 1.5 ng/mL, found no significant difference in outcomes between these two protocols for these patient ($p = 0.16$). In addition, when comparing the patients that had P at surge less than 1.5 ng/mL, we again saw no significant difference in Fresh versus Freeze-all ($p = 0.57$).
Limitations, reasons for caution: Variability in reported values of P due to laboratory assay variation and cycle timing could be a confounding factor in grouping patients with elevated P. Our study was performed on retrospective data from the United States. Future studies could expand to European practice patterns and involve randomized controls trials.
Wider implications of the findings: Freeze-all cycles provide advantages for patients over fresh embryo transfer cycles. We found that not only are pregnancy outcomes equivalent from fresh cycles in younger patients, but that freeze-all protocols provide benefit for older patients. Interestingly, we did not see this in patients with a premature rise in progesterone levels.
Trial registration number: None.

SELECTED ORAL COMMUNICATIONS

SESSION 63: WHAT SPERM CAN DO MORE THAN FERTILIZE

Wednesday 06 July 2016 **Hall 3 AB** **10:00–11:45**

O-240 Isolation and characterization of exosome nanovesicles secreted by pre-pubertal Sertoli cells

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Study question: The aim of our work was to verify if pre-pubertal Sertoli cells (SC) release exosome nanovesicle (Nv) and analyse their mRNA content.
Summary answer: For the first time in the Literature, our work showed the presence of Nv secreted by SC including their mRNA content.
What is known already: Recent studies have shown that extra-cellular secretion of Nv is an important mechanism of intercellular communication. In fact, Nv may contain proteins, DNA and mRNA. In particular, the latter play an important role in various biological processes including regulation and cell differentiation. SC, once described as a mere "sustentacular cell," were re-evaluated in their functions and elevated to the rank of a "sentinel" in spermatogenesis due to production of trophic, differentiation and immune-modulators factors.
Study design, size, duration: Pure porcine pre-pubertal SC were isolated according to previously established methods. Briefly, the testes underwent enzymatic digestion and were cultured in specific SC medium.
Upon 48 h culture, SC were stimulated with FSH or FSH + testosterone (T) and: (a) the presence in the medium of SC-derived exosome nanovesicle (SC-Nv) and (b) SC-Nv content, in terms of Anti-Müllerian hormone (AMH), inhibin B, Androgen Binding Protein (ABP) and FSH-receptor (FSH-r), by Real Time PC, were assessed.
Participants/materials, setting, methods: After 48 h of SC stimulation, culture media were centrifuged at 2000 g (to remove cell debris) and the obtained supernatant underwent further centrifugation with Vivaspin 300 kD at 4000 g for 30 min. Upon washing, the obtained cell pellet was resuspended in both PBS, for scanning electron microscope (SEM) and Dynamic Light Scattering (DLS) analysis, and Trizol, for total mRNA extraction.
Main results and the role of chance: SEM analysis highlighted the presence of SC-Nv in culture medium with 3 different mean diameters (1388, 838 and 149 nm). We have also demonstrated, within the SC-Nv, significant increase in mRNA for AMH, ABP and FSH-r after both FSH and FSH + T stimulation, as compared to unstimulated SC-Nv. Differently from unstimulated SC-Nv, mRNA inhibin B levels were unchanged in FSH-stimulated SC-Nv, and increased after FSH + T stimulation. Interestingly, an opposite trend was shown in mRNA secretion, in control SC monolayer where, we demonstrated a decrease of AMH and FSH-r mRNA (after both stimulation with FSH or FSH + T) and an increase of inhibin B mRNA. On the contrary, mRNA ABP levels, in SC monolayer, decreased after stimulation with FSH but were unchanged in the presence of FSH + T.
Limitations, reasons for caution: These preliminary data will be confirmed in future experiments.
Wider implications **of the findings:** For the first time in the Literature, our work has shown the presence of SC-Nv containing AMH, inhibin B, ABP and FSH-r mRNA. This result may suggest that other testicular cells could produce factors that, until now, were considered an exclusive SC secretory product.
Trial registration number: Not required.

O-241 Decoding mammalian fertility: characterising the genetic loci implicated in male fertility potential

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Study question: The aim of this study was to compile and collate data implicating genes in male fertility, including data demonstrating how particular genes contribute to mechanisms of male reproductive physiology.
Summary answer: We outline the incorporation of comparative genomic data into a systems-level knowledgebase of fertility-centric data to comprehensively understand the molecular drivers of male reproductive physiology.
What is known already: It is estimated that for around a third of couples unable to conceive a child, subfertility of the male partner is the sole explanation. This subfertility remains unexplained for almost half of these men, even after extensive clinical evaluation. Genetic mutation likely accounts for the majority of these patients. However, identification and characterization of the genetic elements collectively contributing to male subfertility is only possible with a fully comprehensive understanding of the genes involved in male-specific reproductive processes.
Study design, size, duration: We collected and cross-referenced human- and animal-model specific expression data, phenotypic and genotypic data for more than 5,000 genetic loci.
Participants/materials, setting, methods: Data were iteratively cross-referenced and analyzed using meta-analyses and bioinformatics tools to characterize roles in particular reproductive processes.
Main results and the role of chance: From >55,000 mammalian phenotypes described on the international database for the laboratory mouse (MGI), a series of algorithmic searches identified 2,493 directly or indirectly linked to reproduction. A second set of algorithms was then used to group and cross-reference genetic loci according to their inferred and/or reported male-specific reproductive function(s). Our algorithmic approach led to the inclusion of more than 1,100 genetic loci in our knowledgebase whose associated phenotypes linked them to at least one aspect of male-specific reproductive biology. For example, the phenotypes of 254 genes demonstrated their role in spermiogenesis, the spermatogenic process involving the morphological differentiation of spermatozoa. Perhaps understandably, 33 of these genes were also reported to result in phenotypes related to sperm count, such as "oligozoospermia." However, our dataset also allowed us to make non-obvious associations between subsets of these 33 genes with endocrinological and immunological phenotypes, thus highlighting potentially important genes and physiological pathways contributing to the etiology of abnormal sperm metrics. Our results contribute to a powerful data set with which to identify candidate genetic biomarkers of human fertility potential, and thus novel targets for drug development and therapeutics.
Limitations, reasons for caution: Data relating particular genes and variants to reproductive processes in mammalian models or humans may have yet to be identified.
Wider implications of the findings: Our classification of genetic loci according to their role in reproductive mechanisms helps clarify how genetic mutations contribute to the pathophysiology of male subfertility. Identifying genetic loci for which little existing information links them mechanistically to infertility, provides a route for hypothesis-driven research into novel, clinically actionable molecular targets.
Trial registration number: None.

O-242 Fingerprinting of tubulin post-translational modifications (PTM) identifies the spermatozoa tail tip as a cluster area of candidate sperm fitness biomarkers

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Study question: What is the make-up and distribution of sperm flagella tubulin PTMs, and their relationship with sperm fitness in the human species?
Summary answer: Human sperm flagella have several PTMs with specific distributions in the last 10% of the tail, which differ between motile and unselected semen fractions.
What is known already: The microtubules in the sperm flagella are composed of tubulin, and are necessary for sperm movement. Tubulin can be modified post translationally by the addition or removal of different amino acids; defining the flagellum functionality. Several tubulin PTMs have been described in *Drosophila* and sea urchin spermatozoa, where blocking tubulin polyglutamylation decreases sperm motility. Tubulin monoglycylation and acetylation are associated with axoneme stability, while preventing tubulin glycylation causes sperm defects and infertility in *Drosophila*. Despite the reported effect of PTMs on sperm fitness, very little is known about PTMs presence and role in human sperm functionality.
Study design, size, duration: This is a basic research study. Fresh ejaculate from normozoospermic and teratozoospermic patients was processed by swim-up. The presence and distribution of the following tubulin PTM were analyzed by western blot (WB) and immunofluorescence (IF) using specific antibodies both in the motile fraction and the unselected one: acetylation, polyglutamylation, branching glutamylation, monoglycylation, lack of the two last amino acids, lack of tyrosine, and tyrosination.
Participants/materials, setting, methods: WBs were carried out on 1.5×10^6 spermatozoa while IF was performed on 100,000 spermatozoa for each condition. A double IF for tubulin and each PTM was performed. The localization pattern of 20 spermatozoa per condition was further analyzed by calculating the tail intensity for every 0.1 micron to then normalize the tail length to 1, and interpolar lineation was applied to calculate the relative intensities across each tail for each 0.001 relative length units.
Main results and the role of chance: We found that the microtubules in the human sperm flagella are highly modified overall; in fact, we detected acetylation, polyglutamylation, branching glutamylation, monoglycylation, lack of the two last amino acids, lack of tyrosine, and tyrosination both by WB and IF. Interestingly, although some of them (lack of the two last amino acids, lack of tyrosine, tyrosination and polyglutamylation) show a similar distribution pattern along the flagella of all normozoospermic and teratozoospermic samples, other (acetylation, monoglycylation and branching glutamylation) are especially reduced in the last 10% of the flagella. Moreover, the specific reduction pattern is different for motile normozoospermic and either unselected normozoospermic and any teratozoospermic sample (motile or unselected), indicating that the motile fraction selected by swim-up of pathological semen, although morphologically normal, could retain some ultrastructural alterations when compared to normozoospermic samples. Overall, our results suggest that tubulin modifications are important for flagellum stability and tail motility, overall affecting sperm functionality.
Limitations, reasons for caution: No attempts to correlate tubulin PTMs and reproductive outcomes were made yet. A degree of phenotype overlap between sperm from the selected and the unselected fractions makes the interpretation of molecular data difficult; samples with close to no motility will be necessary to strongly correlate PTMs fingerprinting with sperm motility.
Wider implications of the findings: This is the first study that describes the presence of most known tubulin PTMs in human sperm. Our results identify the last 10% of the sperm flagellum length as a region of high PTM plasticity. PTM fingerprinting of this area could provide important marker for sperm diagnosis and prognosis.
Trial registration number: N/A.

O-243 Dutasteride mediated morphological changes in genitourinary tract was associated with altered expression patterns of androgen receptor and estrogen receptor in male rat

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Study question: Does dutasteride affect the histological and hormonal status in genitourinary tract other than prostate?
Summary answer: Dutasteride caused the fibrotic changes in penis associated with the altered expression of androgen receptor and estrogen receptor.
What is known already: 5-alpha reductase inhibitors are known to cause adverse effects on sexual function, and some clinical evidences suggest that these adverse effects are persistent or irreversible in some patients. These facts indicate that 5α-RIs may cause the structural degeneration in penis. In fact, Traish et al. reported that finasteride treatment decreased autophagy and produced deterioration in the ultrastracture of the corpus cavernosum, including mitochondria injury and increased trabecular smooth muscle cell death. It is inferred that these changes were caused by altered hormonal environment in the tissue. However the actual hormone dynamics in genitourinary tract related to 5α-RI is still unknown.
Study design, size, duration: Fifteen 8-week-old male Sprague-Dawley rats were used. The animals were divided into 3 groups comprising 5 animals each

and treated as follows. Group A as control group, members of Group B received oral administration of dutasteride 0.1 mg/kg/day from the age of 8 weeks to 12 weeks, and members of Group C were castrated at 8 weeks. All rats were sacrificed at the age of 12 weeks.
Participants/materials, setting, methods: After the measurement of size, bladder, prostate, seminal vesicle, and penis were removed for pathological examination. Smooth muscle/collagen ratio was evaluated by azan staining and measured using color assisted quantitative image analysis to evaluate tissue fibrosis. We also evaluated the expression of androgen receptor and estrogen receptor by immunofluorescent staining.
Main results and the role of chance: The results showed the mean size of prostate and seminal vesicle was smaller in castrated group and dutasteride group than that of control group. Moreover, in castration group, the bladder volume and penile length was also smaller than that of control group. Castration group showed statistically significant histological changes such as increase in fibrotic tissue in bladder, prostate and penis. Similarly, dutasteride group showed fibrotic changes in prostate and penis compared to the control group. Immunofluorescent staining revealed that androgen receptor was more strongly expressed than that of estrogen receptor in control group. On the other hand, in castration group, week expression of androgen receptor and strong expression of estrogen receptor was noted. In dutasteride group, these changes were also noted in prostate and penis. These findings suggest that dutasteride cause morphological changes not only in prostate, but also in penis. These changes are associated with altered expression patterns of androgen receptor and estrogen receptor.
Limitations, reasons for caution: This study only included adolescent rat, therefore, it would be difficult to apply present findings to the entire cohort of male.
Wider implications of the findings: Dutasteride induced changes of hormonal receptor expressions and tissue fibrosis may explain the mechanisms of persistent or irreversible adverse effects of dutasteride in some patients with benign prostate hyperplasia or alopecia.
Trial registration number: None.

O-244 Quantitative proteomic analysis of human sperm tail in asthenozoospermic patients

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Study question: Are there quantitative variations in the sperm tail proteome of asthenozoospermic patients compared to normozoospermic donors?
Summary answer: Compared with normozoospermic donors, 479 proteins were altered in sperm tail proteome of asthenozoospermic patients.
What is known already: The sperm tail is an important structure for the elucidation of the molecular dynamics of sperm motion, as it is the only means of motility and contains mitochondria as the power plants of the sperm. Subcellular proteomics with combining high-throughput techniques allows us to identify low-abundance proteins which would otherwise be undetectable by conventional methods.
Study design, size, duration: This was a case-control study comprising 80 men who attended Royan infertility center for assisted reproduction between October 2014 and July 2015.
Participants/materials, setting, methods: Semen samples were evaluated by computer assessed analyzer and divided into asthenozoospermic group (progressive motility <32%, $N = 40$) and normozoospermic group (progressive motility >32%, $N = 40$). Samples of four individuals were pooled and the tail fractions isolated by sonication and successive sucrose gradient. After confirming tail fraction purity, extracted proteins were labeled with tandem mass tags (TMTs) followed by shotgun proteomics. Bioinformatic analyses were performed using DAVID. Candidate proteins were further validated by Western blot analysis.
Main results and the role of chance: We detected 2145 proteins in the tail fraction of human sperm where 189 and 280 proteins were respectively up and down regulated in asthenozoospermic patients compared to normozoospermic donors. The main down-regulated proteins were structural proteins as well as those involved in energy production pathways. Furthermore, we identified DDX3Y (ATP-dependent RNA helicase), a Y chromosome encoded protein, being over expressed in the sperm of asthenozoospermic patients.
Limitations, reasons for caution: Due to difficulties in isolation of the tail fraction as well as the lack of an adequate amount of protein for shutgun proteomics, samples from four individuals were pooled.
Wider implications of the findings: While it is known that Y chromosome encoded proteins play a critical role in male gonad development and spermatogenesis, our data highlight their significance in the function of mature spermatozoa.
Trial registration number: 0000.

O-245 Signaling pathways in human spermatozoa to screen for idiopathic infertility

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Study question: We question whether the gene products compartmentalized in the human spermatozoon influence semen parameter characteristics and if the specific genes involved effect embryo developmental competence.
Summary answer: Expressivity of the genes whose protein is localized in various components of the sperm cell provides inherent information on male gamete competence.
What is known already: The limitation of evaluating human semen characteristics, even according to the most stringent criteria, is known to provide limited information on the performance of spermatozoa even when used for assisted reproductive technologies (ART). Standard semen analysis is ill-defined in predicting reproductive outcome in men with idiopathic infertility. For this purpose, attempts have been made to detect ploidy, chromatinic integrity, highly detailed morphology and maturational status of the spermatozoon. Epigenetic assessment by profiling RNA transcripts in the sperm cell has been proposed as an alternative method to screen the male partner for infertility.
Study design, size, duration: In a 12-month period, we assessed the eventual impact of gene products specific to the sperm cell on semen characteristics and reproductive outcome. RNA extraction was carried out on 26 semen specimens of consenting men undergoing infertility screening. The specific proteins that were allocated among key structural components of the spermatozoon, such as the acrosomal vesicle, nucleus & flagellum were compared to sperm concentration, motility, morphology, as well as embryonic development and implantation.
Participants/materials, setting, methods: An average of 25×10^6 human spermatozoa was used to isolate total RNA using a spin column commercial kit. The nucleic acid quality and spermatozoal RNA concentration was assessed. The RNA samples were then made into paired-end libraries. Pilot paired-end 76 bp RNA-Sequencing (RNA-Seq) using an Illumina platform (NextSeq 500) was carried out and expanded to 60M reads. Expression values were calculated in Fragments Per Kilobase Of Exon Per Million Fragments Mapped (FPKM) and normalized read counts.
Main results and the role of chance: From a total of 26 men that provided sperm for RNA extraction, 8 were chosen for their adequate nucleic acid quality and concentration. Total RNA extraction from an average of 25×10^6 spermatozoa (range 4×10^6–30×10^6) was utilized. These men had an average age of 26 ± 5 years and presented with a sperm concentration of 27.3 ± 27, a motility of 46.6 ± 24, and a morphology of 3.0 ± 2. After grouping gene expression to their respective spermatozoon compartment, *ATP6V1E2* localized to the acrosomal vesicle, *TSSK6 & H1FNT* localized to the nucleus, *AKAP4 & CATSPER1* localized to the flagellum, *AGPAT2* localized to the endoplasmic reticulum, and *PLK4* localized to the centrosome. After plotting the expression in FPKM of all the above mentioned genes against the semen parameters *AKAP4,* a gene that encodes a protein localized to the sperm flagellum, had a strong inverse correlation with motility ($P = 0.01$). When ART outcome was evaluated, *ATP6V1E2* (acrosomal vesicle) had an inverse correlation to fertilization rates ($P = 0.03$). All gene products localized in the head and midpiece (centriole) were strongly expressed in men that established pregnancy that resulted in delivery ($P = 0.01$).

Limitations, reasons for caution: This study investigates the ability to profile men with idiopathic infertility and must be confirmed in a larger cohort. While the contribution of the female partner cannot be excluded, gene expression profiling of infertile men may serve as an assay to measure the reproductive potential of the male gamete.
Wider implications of the findings: Deep sequencing of sperm RNA is a reliable and reproducible technique that may aid in the diagnosis and screening of infertile men. Gene products specific of the diverse compartments of the sperm cell may supplement standard semen parameters and provide a pivotal tool in the diagnosis of idiopathic infertility.
Trial registration number: N/A.

O-246 The impact of sperm protamination on embryo development and ART result

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Study question: The percentage of histone protein remaining in spermatozoa chromatin could be calculated and used as a prognostic factor of fertilizing capacity and a prognostic factor for ART success?
Summary answer: The HRI is partially correlated to sperm DNA fragmentation. A relationship with embryo development has been found. HRI indirectly influences the ART success.
What is known already: The Histone Protamine Ratio (HRI) is the spermatozoa protamination reflect. The protamine ratio (P1/P2) is known to be associated with male factor infertility. However, persistent histones play an important role in the sperm epigenome. So an abnormal percentage of remaining histones could impact on embryo development and decrease the rate of ART success.
Study design, size, duration: A prospective study was performed at the HFME Hospital (Bron, France). A total of 291 ART cycles were included. ART procedure occurred between October 2013 and July 2015. The exclusion factors were: less than 6 oocytes punctured, cryopreserved sperm used and a total number of spermatozoa below 40 million.
Participants/materials, setting, methods: In order to calculate the HRI, a modified method described by Wikes (2003) was used. Modifications were done in order to obtain a reproducible procedure. DFI was measured with TUNEL technique. The measurement were performed on sperm used for ART procedure. A hierarchical analysis was performed to calculate the HRI cut-of value that allows obtaining the higher pregnancy rate (PR).
Main results and the role of chance: 42 c-IVF and 249 ICSI procedures were performed. 3870 oocytes were punctured, 2211 embryos were obtained: 307 embryos with c-FIV procedure and 1904 embryos with ICSI procedure. Among those embryos, 507 were transferred and 336 were frozen. The mean HRI was equal to 18.9%. The mean HRI find in this study corresponds to the remaining histone as usually described in human sperm. A significant negative correlation coefficient was fount between HRI and DFI ($r = -0.12$, $p < 0.05$). When the HRI was within the range (6–26%), the probability to obtain a blastocyst was higher: 87.8%, against 71.2% for HRI below 6 and 75.7% for HRI above 26% ($p < 0.01$). The highest PR: 27.8%, was obtained for HRI within the range (6–26%), the PR was equal to 25.0% for HRI below 6%, equal to 20.9%, when HRI was 26%; however the differences were not statistically significant.
Limitations, reasons for caution: The size of the sample should be increased in order to confirm the relationship between HRI and pregnancy. The method to quantitate HRI should be adapted to low number of spermatozoa, as actually only cycles with good sperm could be studied.
Wider implications of the findings: This procedure allows a reliable evaluation of the sperm protamination. There is a relationship between HRI, embryo development and ART success. Another proof of the implication of male factor on embryo development was found. The HRI seems to be more predictive than DFI and could be included in sperm investigation.
Trial registration number: Agence de Biomédecine: R12109CC/RPD12001CCA.

SELECTED ORAL COMMUNICATIONS

SESSION 64: SAFETY AND QUALITY IN ART 2

Wednesday 06 July 2016 **Hall 3 DE** **10:00–11:45**

O-247 Which patient and treatment factors contribute to the risk of congenital heart defects after art?

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Study question: Do congenital heart (CH) defects occur with elevated frequency after ART, and how are patient factors and treatment modality associated with the risk?
Summary answer: Maternal factors contributed to all 3 major ICD-9 classes of CH defect. Specific ART treatments were associated with 2 of the 3 classes, after adjustment.
What is known already: CH defects occur in around 8 per 1,000 births and are a leading cause of death in the first year of life. Approximately half of children diagnosed will require surgery, with more complex conditions often requiring lifelong medical care. We and others have reported previously that ART is associated with an increased risk of birth defects. There is a need to consider specific defect types by mode of treatment, together with a range of patient characteristics, in a representative sample.
Study design, size, duration: Cohort study of all deliveries ($n = 302,811$) and terminations of pregnancy in South Australia for the period Jan 1986–Dec 2002 that were linked to all cycles of ART (6,163 births) for the same time period. These data were linked to a State-wide birth defect registry.
Participants/materials, setting, methods: The South Australian Birth Cohort is based on all registrations of birth and terminations of pregnancy, linked to all cycles of ART, and to all congenital anomalies notified to the 5th birthday (ICD-9 British Paediatric Association codes). Logistic regression was used to investigate associations between parental factors, treatment modality and the presence of CH defects.
Main results and the role of chance: Compared to women aged 25–29 years, those aged 35–39 and 40–45 years had greater risk of having a child with CH defect (an increase of 18 and 48%, respectively). Nulliparous women had an elevated risk of CH defect in the child of 9%. Diabetes and hypertension each increased the risk of CH defect by approximately 30%, and a history of miscarriage increased risk by approximately 10%. Over time, CH defects have been trending down, by around 5% per year. Female babies had a higher risk of defect overall compared to males, an increase of 7%. Twin pregnancy increases the risk of CH defect, by around 40%. ART singletons carried the same risk as twins.
Compared to the fertile population: (a) Cardiac Septal Closure anomalies (BPA 74500–74599) did not vary by ART treatment, but were increased amongst "natural" conceptions to ART patients, and after infertility consultations conducted outside an ART clinic.

(b) Other Congenital Heart anomalies (BPA 74600–74699) were increased for fresh IVF (OR = 1.9), IUI (OR = 2.3) and "natural" conceptions to ART patient (OR = 2.2) (c) Other Congenital Circulatory System anomalies (BPA 74700–74799) were increased for IVF fresh (OR = 2.0) ICSI frozen (OR = 4.7) IUI (OR = 2.4) and fertility consultation outside an ART clinic (OR = 2.5).
Limitations, reasons for caution: The decline in CH defects over time mean that more recent data are needed. We are also limited in power, with a likelihood of having false negative findings in some comparisons. We do not have access to specific treatments received outside ART clinics where ovulation induction is common.
Wider implications of the findings: Cardiac septal closure defects were related to maternal factors and non-ART infertility care.

Other anomalies of the heart and circulatory system varied significantly by ART treatment. Neither singleton pregnancy nor use of embryo cryopreservation reduced the excess risk of cardiac defects. Further basic research is therefore indicated.
Trial registration number: Not applicable.

O-248 Trends over time in congenital malformations among children conceived after assisted reproductive technology (ART)

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Study question: Has there been a change in the risk of congenital malformations for children conceived after ART during latest decades?
Summary answer: In contrast to the declining rates of poor neonatal outcomes, the incidence of major congenital malformations among ART children has remained stable over time.
What is known already: Recently, it has been demonstrated that the risk of several adverse perinatal outcomes, has decreased over time for both singletons and twins. Children conceived after ART have a well-known increased risk of congenital malformations compared to spontaneously conceived (SC) children, but whether the rate is declining over time as other neonatal risks has not yet been explored. Some studies have found a particularly increased risk of congenital malformations for children conceived after intracytoplasmic sperm injection (ICSI), which still needs further investigation.
Study design, size, duration: Population-based cohort study including 90 201 ART children and 482 552 SC children. Both singletons and twins born after IVF, ICSI and frozen embryo transfer were included. The ART singletons were matched 1:4 on with a control group of SC children from their own country. The matching criteria were parity (0 versus 1) and year of birth. To allow stratified analyses for twins, all SC twins born within the study period were included.
Participants/materials, setting, methods: Multiple logistic regression analyses were used to estimate the risks and adjusted odds ratios for congenital malformations in four time periods: 1988–1992; 1993–1997; 1998–2002 and 2003–2007. To assess whether the risk patterns between the conception groups changed over time we tested group-time interaction terms. All malformations were classified and grouped according to the EUROCAT classification of malformations. Only major malformations were included. We used nationwide health registers from Denmark, Finland, Norway and Sweden.
Main results and the role of chance: A major congenital malformation was observed among 2304 (3.76%) ART versus 11 110 (3.17%) SC singletons, adjusted odds ratio (AOR) 1.15 [95% confidence interval (CI), 1.10–1.21]. Among twins, $n = 1785$ (6.17%) ART versus $n = 6970$ (5.29%) SC twin children had a major congenital malformation, AOR 0.93 (95% CI, 0.88–0.99). For all ART children the total risk of any major congenital malformation was AOR 1.14 (95% CI, 1.10–1.18). The difference in risk of major malformations between children conceived after ART and SC did not change significantly during the four time periods, neither for singletons, $p = 0.46$; nor twins, $p = 0.30$. The risk of any major congenital malformation was the same for all children conceived after IVF versus ICSI, AOR 1.00 (95% CI, 0.92–1.08). This was also the case regarding the risk of urinary malformations, AOR 0.97 (95% CI, 0.71–1.33) and genital malformations, AOR 0.76 (95% CI, 0.57–1.00). When comparing children born after replacement of a fresh embryo with children born after replacement of a frozen-thawed embryo, no difference in risk of any major malformation could be detected, AOR 1.06 (95% CI, 0.95–1.19).
Limitations, reasons for caution: Many factors potentially affect the development of the early embryo. Residual confounding by parental factors is possible and the Nordic ART population has changed over time. Although no clear differences in risk of any major malformation were found between specific ART procedures, a contribution from treatment factors cannot be excluded.
Wider implications of the findings: Since the incidence of major congenital malformations remained stable over time, this may indicate that an increased risk for ART children persists in spite developments in both clinical programs and laboratory techniques over time. Still, the total risk of congenital malformations in ART children remains slightly elevated.
Trial registration number: 0.

O-249 The influence of specific infertility treatments on long-term cognitive abilities in children: a systematic review

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Study question: Are children conceived with infertility treatment at increased risk of impaired cognitive development in the long-term, and does the type of treatment alter the risk?
Summary answer: Although a substantial literature on longer-term cognitive outcomes exists, most studies have methodological limitations. Population-based studies are required to clarify the impact of specific treatments.
What is known already: There have been longstanding concerns about the extent to which infertility treatment alters programming of early development, and whether this has enduring consequences for children's cognitive function. Previous reviews of this literature have generally concluded that overall, cognitive outcomes are "comparable" between children conceived with infertility treatment and those conceived naturally. However, in the existing reviews there has been varying assessment of the quality of available studies. In addition, none have separately reviewed the range of components involved in infertility treatment, to determine whether specific aspects (mode of conception, cryopreservation, donor gametes etc.) have different effects on neurodevelopment.
Study design, size, duration: We undertook a systematic review of studies published in English before 5th January 2016. Studies were eligible if they assessed cognitive development from age 5 years or more, among children conceived with infertility treatment compared with either children conceived naturally or children born from a different type of infertility treatment. A meta-analysis was not performed as the included studies used a wide range of cognitive assessments.
Participants/materials, setting, methods: We searched the databases PubMed, PsycINFO, and ERIC, as well as the reference lists of included studies. Two authors independently reviewed papers for eligibility, extracted data and assessed study quality. Where available, data were extracted and reported separately according to the mode of conception, exposure to cryopreservation, use of donor gametes, and the type of comparison group used. Risk of bias was assessed using the Newcastle–Ottowa Scale, with a score ≥7/9 indicative of high quality.
Main results and the role of chance: Of 845 publications identified, 33 were eligible. Seven studies were high quality. Among moderate/poor quality studies the main limitation was selection bias, resulting from exclusion of children at increased risk of intellectual impairment (23 studies) or use of discrete clinic populations (26 studies). Among high quality studies, there were no differences in cognitive abilities between IVF children compared with natural conceptions (Table 1). In contrast, findings among ICSI children were inconsistent. Studies assessing exposure to cryopreservation were scant.

Table 1. Number of included studies and findings of high quality studies.

Comparison	No. of studies (No. high quality)	Findings of high quality studies
Any treatment vs. NC	27 (7)	4 studies: ↑ risk of cognitive impairment in treated group; 3 studies: #
IVF vs. NC	11 (3)	#
ICSI vs. NC	13 (3)	1 study: ↓ IQ among ICSI children; 2 studies: #
Cryopreservation vs. NC	1 (1)	#
OI alone vs. NC	2 (1)	↑ risk of mental disorders with OI

Comparison	No. of studies (No. high quality)	Findings of high quality studies
Donor gametes vs. NC	3 (1)	#
IVF vs. ICSI	9 (3)	2 studies: ↓ IQ among ICSI children; 1 study: #
IVF/ICSI vs. NC in subfertile couples	8 (1)	#

NC, natural conceptions; #, no difference.

Limitations, reasons for caution: We undertook a systematic search across a range of databases to identify relevant studies, and two authors independently made decisions about study eligibility as well as study quality. Nevertheless, it is possible that relevant studies may have been missed.
Wider implications of the findings: There are gaps in knowledge about the longer-term impact of certain aspects of treatment including ICSI and cryopreservation. Further studies are indicated, that include the complete group of children exposed to treatment, and utilise appropriate comparison groups to discern the effects of treatment and parental health characteristics.
Trial registration number: Not applicable.

O-250 Long-term prognosis of live birth after ART, intrauterine insemination and spontaneous conceptions in women initiating treatment with homologous gametes – a Danish national cohort study

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Study question: What is the prognosis of live birth 2, 3 and 5 years after first treatment, and how many deliver after assisted reproductive technology (ART), intrauterine insemination (IUI) and spontaneous conception?
Summary answer: Overall, 71% of the women had a live birth within 5 years from the first treatment, of which 57% conceived after treatment and 14% conceived spontaneously.
What is known already: Cumulative birthrates have been reported as birthrates after a number of complete ART-cycles, where one cycle refers to all fresh and frozen-thawed transfers, generated from one oocyte pick-up. Although reflecting the efficiency of ART, it may not be the optimal measure when advising couples, since the time-frame of one cycle can vary considerably. Couples may shift between ART and intrauterine insemination, but few studies include IUI-treatments. Choice of statistical method influences the estimated birthrates substantially. Survival analyses assume that subjects who discontinue treatment have the same prognosis as subjects who continue, whereas conservative rates reflect the proportion who actually delivers.
Study design, size, duration: This cohort study includes all women initiating fertility treatments with homologous gametes in public and private clinics in Denmark 1997–2010, $N = 19\,884$. Subjects were identified in the Danish ART Registry and treatment-cycles were cross-linked with the Medical Birth Registry to identify live birth after treatment and spontaneous conception. Subjects were followed 2, 3 and 5 years or until the first live birth. Women were censored after the first live birth and if they shifted to donor semen.
Participants/materials, setting, methods: Cumulative live birthrates were calculated as the number of women with at least one live birth 2, 3 and 5 years after the first ART or IUI-cycle, divided by the total number of women entering treatment, including also spontaneous conceptions (SC). Birthrates were subdivided according to mode of conception (ART/IUI/SC), and stratified according to type of first treatment (ART/IUI). Birthrates were also stratified according to female age at time of first treatment.
Main results and the role of chance: Complete 2, 3 and 5 years follow-up was available for 19,884 women, 14,445 women and 5165 women, respectively. The total live birthrates 2, 3 and 5 years after treatment were: 57.0% (95% CI 56.3–57.7), 65.0% (95% CI 64.2–65.8) and 71.0% (95% CI 69.8–72.2).

In women where ART was the first treatment, 46.1% (95% CI 45.0–47.2), 51.1% (95% CI 49.8–52.4) and 52.9% (95% CI 50.8–55.0) delivered after ART-conception within 2, 3, and 5 years. In this group, the birthrates for spontaneous and IUI-conceptions after 5 years were 11.2% (95% CI 9.9–12.5) and 0.6% (95% CI 0.3–0.9), respectively.

When the first treatment was IUI, 34.2% (95% CI 33.4–35.0) delivered after IUI-conceptions within 2 years, and the birthrate did not increase significantly after 3 and 5 years. Shift to ART-treatment resulted in birthrates for ART-conceptions of 15.1% (95% CI 14.5–15.7), 21.1% (95% CI 20.3–22.0) and 23.7% (95% CI 22.2–25.2), after 2, 3, and 5 years respectively. After 5 years, 16.6% (95% CI 15.3–17.9) of women starting treatments with IUI had delivered after spontaneous conception.

Age-stratified analysis at 5 years follow-up in women <35 years, 35–40 years and ≥40 years: the total birthrates were 80.0% (95% CI 78.9–81.1), 60.5% (95% CI 59.2–61.8) and 26.2% (95% CI 25.0–27.4), respectively.
Limitations, reasons for caution: The national ART register is compulsory and clinics should report all initiated treatment-cycles, but underreporting of cycles not resulting in a pregnancy may occur. Since the register is cycle-based and most women have repeated treatments, it is less likely that we have underestimated the number of women with no birth.
Wider implications of the findings: Based on these results, we are now able to provide couples with a comprehensible, age stratified long-term prognosis at start of treatment. Birthrates after IUI did not increase after 2 years, which reflect the Danish treatment strategy: 3–6 cycles of IUI followed by ART.
Trial registration number: The study was approved by the Danish Data Protection Agency (J. nr. 2012-41-1330).

O-251 Perinatal outcomes following preimplantation genetic diagnosis versus IVF or ICSI: analysis of 99,498 singleton live births

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Study question: Does embryo biopsy for preimplantation genetic diagnosis (PGD) affect perinatal outcomes such as preterm birth (PTB) and low birth weight (LBW).
Summary answer**:** There was no increased risk of PTB, early PTB, LBW and very LBW following PGD suggesting that embryo biopsy did not influence these perinatal outcomes.
What is known already: Pregnancies resulting from assisted reproductive treatments (ART) are associated with a higher risk of pregnancy complications compared to spontaneously conceived pregnancies. The possible reason of adverse obstetric outcomes following ART has been attributed to the underlying infertility itself and embryo specific epigenetic modifications due to the *in vitro* fertilisation techniques. It is of interest whether interventions such as embryo biopsy as performed in PGD affects perinatal outcomes.
Study design, size, duration: Anonymous data were obtained from the Human Fertilization and Embryology Authority (HFEA), the statutory regulator of assisted reproduction treatment (ART) in the UK. The HFEA has collected data prospectively on all ART performed in the UK since 1991. Data from 1991 to 2011 involving a total of 99,498 singleton live births (452 following PGD cycles and 99,046 following stimulated fresh IVF ± ICSI cycles) were analysed.
Participants/materials, setting, methods: Data on all women undergoing stimulated fresh IVF ± ICSI or PGD treatment were analysed to compare PTB, early PTB, LBW and very LBW. Occurrence of a live birth at <37 weeks gestation is defined as PTB and <32 weeks gestation as early PTB. Birth weight <2500 g is defined as LBW and <1500 g as very LBW. Logistic regression analysis was performed adjusting for female age, previous IVF cycles, infertility diagnosis, oocyte number, IVF or ICSI.
Main results and the role of chance: The unadjusted odds were OR 0.66, 95% CI 0.46–0.97 for PTB, OR 0.58, 95% CI 0.24–1.40 for early PTB, OR 0.58, 95% CI 0.38–0.86 for LBW and OR 0.61, 95% CI 0.25–1.48 very LBW. After adjusting for potential confounders such as female age, previous IVF cycles, diagnosis of infertility, number of oocytes retrieved and IVF or ICSI, there was no significant increase in the risk of adverse perinatal outcomes following PGD: PTB [adjusted odds ratio (a OR) 0.70, 95% CI 0.48–1.03], early PTB (a OR 0.52, 95% CI 0.19–1.38), LBW (a OR 0.59, 95% CI 0.44–1.05) and very LBW (a OR 0.51, 95% CI 0.91–1.39).
Limitations, reasons for caution: Although the analysis was adjusted for a number of important confounders, the dataset had no information on confounders such as smoking, body mass index and medical history of women during pregnancy to allow adjustment. It should also be noted that majority of PGD cycles in the dataset involved blastomere biopsy.

Wider implications of the findings: Analysis of this large dataset of singletons following PGD and IVF ± ICSI suggests that there is no increased risk of adverse perinatal outcomes following PGD. Embryo biopsy for PGD did not introduce extra perinatal risks. This information is useful for counselling patients.
Trial registration number: Not applicable.

O-252 Follow-up of children born after new technologies: testicular sperm extraction (TESE), *in vitro* maturation (IVM), and artificial oocyte activation (AOA)

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Study question: We evaluated the outcome of infants using relatively new technologies –TESE, IVM, and artificial oocyte activation (AOA) – compared with those achieved with conventional IVF.
Summary answer: There were no significant differences between conventional IVF (C-IVF), ejaculated sperm ICSI (ej-ICSI) and advanced technology such as TESE, IVM and AOA.
What is known already: Assisted reproductive technology (ART) has been performed widely and its treatment methods have become complex. In order to verify the safety of new technology, an ongoing follow-up of children is needed.

Some reports have been published about the safety of IVF, ICSI and frozen embryo transfer. However, there are few reports about advanced technologies.
Study design, size, duration: The subjects were 2977 children who were born after fresh or frozen embryo transfer, from October 1995 to October 2014. The subjects were separated into 5 groups: Group 1, conventional IVF; Group 2, ej-ICSI; Group 3, TESE; Group 4, IVM; and Group 5, AOA (using Ca ionophore or $SrCl_2$).
Participants/materials, setting, methods: Among 2977 children [Group 1 (587 singletons, 108 twins, 18 triplets), Group 2 (1614 singletons, 302 twins, 30 triplets), Group 3 (178 singletons, 46 twins), Group 4 (26 singletons, 6 twins), and Group 5 (51 singletons, 8 twins, 3 triplets)], we evaluated perinatal outcomes and their development.
Main results and the role of chance: From Groups 1–5, premature birth rates of singletons were 7.8, 7.0, 4.5, 13.7 and 24.0%. Low birth weight rates of singletons were 10.8, 10.7, 8.5, 11.8 and 12.0%. Abnormality rates were 3.6, 3.8, 1.8, 3.2 and 3.1%, respectively. There were no significant differences between standard IVF and the other groups for perinatal outcomes. In each group, the most commonly recognized congenital abnormality was inguinal hernia due to low birth weight, and the majority of children have recovered naturally. Chromosomal abnormalities – 13 (1, 0), 18 (2, 0) and 21 (5, 1) trisomy were observed in group 2 and group 5, respectively.

Moreover, there were no significant differences in physical or cognitive development up to the age of 6, including weight and height, between standard IVF and the other groups.
Limitations, reasons for caution: None.
Wider implications of the findings: We concluded that advanced technology such as TESE, IVM, and AOA does not increase perinatal risk or affect the growth of children. However, we need to continue long-term follow-up to evaluate more cases.
Trial registration number: None.

O-253 Cumulative live birth rate and perinatal outcomes comparing women with freeze-all embryo transfers and women with fresh and subsequent frozen embryo transfers

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Study question: Are cumulative treatment outcomes better for women who have all embryos frozen before transfer than for women with both fresh and frozen embryo transfers?
Summary answer: Treatment outcomes are similar for women who have all embryos frozen before transfer and those who have both fresh and frozen embryos transfers.
What is known already: Cycle-based assisted reproductive technology (ART) data consistently report that fresh embryo transfers have a higher live birth rate than frozen/thawed embryo transfers. This may be explained by the selective transfer of good embryos in the fresh cycles. However, births following frozen embryo transfers have better perinatal outcomes than those following fresh embryo transfers. It is unknown whether freezing all embryos adversely affects the cumulative live birth rate.
Study design, size, duration: A population-based retrospective cohort study used ART data collected by the Victorian Assisted Reproductive Treatment Authority (VARTA). This study included 10,553 women undergoing their first autologous oocyte pick-up during 1 July 2009 and 30 June 2013 in Victoria Australia. Treatment and pregnancy/birth outcomes were recorded from the start of the first oocyte pick-up through associated thaw cycles until 30 June 2014, or until all embryos used or a live birth achieved.
Participants/materials, setting, methods: Women who had all embryos frozen and subsequently transferred (freeze-all group, $n = 701$) and those with fresh and frozen embryo transfers (fresh group, $n = 9852$) were identified. Groups' treatment characteristics, cumulative live birth rate and rates of adverse perinatal outcomes (preterm birth, low birth weight) were compared. Life-table method was used to calculate the cumulative live birth rate per number of transfer cycles. A generalised estimating equation model was used to assess each adverse perinatal outcome.
Main results and the role of chance: The mean age of women was lower (33.4 years) in the freeze-all than in the fresh group (34.9 years) ($p < 0.01$). About 60% of women in the freeze-all group had >15 oocytes collected compared with 18.5% in the fresh group ($p < 0.01$). In the freeze-all group, 576 women returned for an embryo transfer. The live birth rate following the first transfer was 23.0% in the freeze-all and 25.2% in the fresh group (NS). After six transfers, the cumulative live birth rate was (40.1%) in the freeze-all group, not different from the fresh group (38.2%) ($p = 0.24$). Of singletons born after the first transfer, 7.1% were born preterm in the freeze-all and 8.9% in the fresh group ($p = 0.30$). The low birth weight rate was 5.2% in the freeze-all and 7.0% in the fresh group ($p = 0.28$). For women with ≤15 oocytes collected, the cumulative live birth rate was 22.2% in the freeze-all and 34.3% in the fresh group ($p = 0.55$). For women with >15 oocytes collected, the cumulative live birth rate was 53.0% in freeze-all and 55.6% in fresh group ($p = 0.90$). After adjusting for the number of oocytes collected there were no statistically significant group differences in cumulative live birth or adverse perinatal outcome rates.
Limitations, reasons for caution: Potential confounders such as stimulation protocol and culture media are not collected in the dataset and may have affected the findings of this study. Selection bias is likely given the baseline differences between the two groups. Data was not collected on reason for the initial transfer plan.
Wider implications of the findings: This study suggests that frozen only transfers are not adversely affected which is reassuring for those women who have clinical indications not to have a fresh transfer. A randomized control trial is required to avoid the likely selection bias in this study.
Trial registration number: N/A.

SELECTED ORAL COMMUNICATIONS

SESSION 65: ENDOMETRIOSIS IN THE CLINIC

Wednesday 06 July 2016 **Room 101** **10:00–11:45**

O-254 Increased pain responses and pain symptoms persist until premenopause in women with endometriosis – a population-based cohort analysis

S. Vuontisjärvi[1,2], H. Rossi[1,2], S. Herrala[3], L. Morin-Papunen[1,2], J. Tapanainen[1,2,4], J. Karppinen[2,3,5], J. Auvinen[3], T. Piltonen[1,2]
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Study question: Is the history of endometriosis associated with an altered pain sensation and musculoskeletal (MS) pain symptoms at premenopausal age?
Summary answer: Women with history of endometriosis had lower pain threshold, lower maximum pain tolerance and a higher likelihood of MS pain symptoms at late premenopausal age.
What is known already: Thirty percent of women with endometriosis experience chronic pelvic pain (CPP) and up to 75% of adolescent girls with CPP or dysmenorrhea have laparoscopic evidence of endometriosis. Previous studies have shown increased pain sensitivity in fertile aged women with endometriosis in response to mechanical stimuli compared to non-affected women. Furthermore, pain threshold studies have observed hyperalgesia at extra-pelvic sites in young women with endometriosis. Up to date, population-based studies investigating the association of endometriosis with pain sensation and pain symptoms are lacking.
Study design, size, duration: In a prospective follow-up of a large national birth cohort, a postal questionnaire including questions on previously diagnosed endometriosis, number of pain areas and pain intensity was sent to 5080 women (72% response rate) at 46 years. Of all women, 284 reported having endometriosis whereas others (n = 3390) were defined as controls. A total of 2704 women also participated in a clinical examination assessing pressure pain threshold (PPT) and maximal pain tolerance (PPTo).
Participants/materials, setting, methods: 282 women with endometriosis and 3057 controls answered questions on MS pain, while pain sensitivity measurements were conducted for 234 endometriosis cases and 2470 controls. PPT/PPTo were measured in four sites (shoulder, tibialis anterior muscle, wrist joint, L5/S1 interspinous space) using an algometer (Somedic, Sweden). The association between endometriosis and PPT/PPTo (mean of four sites) was estimated using regression analysis. The results were adjusted for BMI, smoking, depressive/anxiety symptoms, education and use of hormonal contraceptives.
Main results and the role of chance: Women with endometriosis reported more pain sites than the controls: 0 areas for 9.6% of women with endometriosis vs. 17.9% of controls; 1–4 areas for 16.4 vs. 15.8%; 5–8 areas for 6.2 vs. 4.8%, $p < 0.001$. Furthermore, endometriosis was associated more often with disturbing pain at leisure time, during sleep and at work ($p = 0.02$, $p = 0.04$, $p = 0.01$, respectively). The women with endometriosis also reported higher pain intensity compared with controls ($p = 0.02$).

Pain sensitivity assessments showed that women with history of endometriosis had lower PPT (−34.0 kPa, $p < 0.05$) and PPTo (−48.2 kPa, $p < 0.01$) than the controls. Inclusion of all confounding factors in the analyses did not change significantly the strength of associations.
Limitations, reasons for caution: The diagnoses of endometriosis were self-reported. However, 37.7% of cases were available for validation using hospital patient records. The hospital register data confirmed endometriosis diagnosis for 76.3% of self-reported cases and of them 90.1% were diagnosed with laparoscopy.
Wider implications of the findings: This unique population-based data showed a higher likelihood of MS pain and altered pain sensation in women with endometriosis at 46 years. This implies that endometriosis has long lasting consequence on a woman's life as the women may be more prone to MS pain symptoms beyond fertile age.
Trial registration number: –

O-255 Laparoscopic excision versus ablation for endometriosis-associated pain – a systematic review and meta-analysis

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Study question: To systematically review and summarize all existing evidence related to the impact on endometriosis-associated pelvic pain when treated by laparoscopic excision or laparoscopic ablation/vaporisation.
Summary answer: Laparoscopic excision is associated with significantly better reduction in symptoms of dysmenorrhea, dyschezia and chronic pelvic pain compared with laparoscopic ablation/vaporisation in endometriosis.
What is known already: The evaluation and treatment of endometriosis has evolved in recent decades. It is recommended where possible to see and treat the lesions as there is evidence that their removal reduces endometriosis associated pelvic pain and improves spontaneous fertility. A recent Cochrane review concluded that there was low quality evidence that laparoscopic excision and ablation were similarly effective in relieving pain. However this review included only one trial. This study sought to systematically re-review and summarize all existing evidence related to the impact of laparoscopic excision on endometriosis-associated pelvic pain compared with laparoscopic ablation to update and further guide clinical practice.
Study design, size, duration: We searched MEDLINE (1950 to October 2014), EMBASE (1980 to October 2014), ISI conference proceedings, databases for registration of ongoing and archived RCTs, namely International Standard Randomised Controlled Trial Number (ISRCTN), Register and Meta-register for RCTs (http://www.controlled-trials.com), WHO trials search portal (ICTRP, apps.who.int/trialsearch/Trial); the Cochrane Library and the British Library of electronic theses online service (http://ethos.bl.uk) with the search term of "endometriosis." No language restriction was placed. Only RCTs were included.
Participants/materials, setting, methods: Studies were selected if the target population were women undergoing laparoscopic surgery for endometriosis with excision technique and were compared with women with an ablative/vaporisation technique. The primary outcome measure was reduction in dysmenorrhea (the most common pain symptom in endometriosis). Secondary outcomes were reduction in symptoms such as dyspareunia, chronic pelvic pain, dyschazia by VAS and improvement in QOL scores. Statistical analyses were performed using RevMan 5.2.7 software (Cochrane Collaboration, Oxford, UK) and StatsDirect.
Main results and the role of chance: Three RCTs included in this review with 222 participants. 114 women were randomised to treatment with excision and 108 women to ablation. Pooling of the results of these showed that the excision group had significantly more reduction in symptoms of dysmenorrhea (MD 0.99; 95% CI 0.00, 1.98; $p = 0.05$) and dyschezia (MD 1.31; 95% CI 0.33, 2.29; $p = 0.009$) as compared to those with ablation/vaporisation. There was no significant difference in reduction in VAS score for dyspareunia between the two groups (MD 0.96; 95% CI 0.07, 1.99; $p = 0.07$) even though there was a trend towards more reduction with the excision. Only one study reported on reduction in EHP30 Core pain score and reduction in VAS score for chronic pelvic pain and showed that the excision group had significantly more reduction in EHP30 Core pain scores (MD 13.20; 95% CI 3.70, 22.70; $p = 0.006$) and VAS score for chronic pelvic pain (MD 2.57; 95% CI 1.27, 3.87; $p = 0.0001$) compared with ablation/vaporisation. One study reported on reduction in pelvic pain and showed that there was no significant difference in the excision and ablation groups (MD – 0.10; 95% CI 1.30, 1.10; $p = 0.87$).
Limitations, reasons for caution: This systematic review found only 3 RCTs with overall population of only 222 women. The data could be pooled from only 2 RCTs for the meta-analysis. Some of the outcomes were reported only in one study, such as chronic pelvic pain which was clearly defined in only one study.
Wider implications of the findings: From the limited evidence available, Laparoscopic excision is associated with significantly better reduction in symptoms of dysmenorrhea, dyschezia and chronic pelvic pain compared to laparoscopic ablation in cases of endometriosis. Further larger trials are necessary to substantiate these results.
Trial registration number: None required.

O-256 Women with endometriosis do not have an increased miscarriage rate but suffer significantly more from endometriosis associated infertility

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Study question: Is the abortion rate in endometriosis women increased?
Summary answer: The abortion rate is not increased but the psychological distress following miscarriage is higher.
What is known already: Endometriosis is a chronic disease occurring in up to 10% of women during reproductive age. Compared to women without endometriosis the number of childbirths is reduced but can be augmented by artificial reproductive therapy (ART). Ovarian response and oocyte quality are discussed to be diminished in women with endometriosis but once implantation is achieved, the risk of miscarriage is not likely to be increased – current literature does not provide enough evidence for an association between endometriosis and miscarriages.
Study design, size, duration: Retrospective case control observational study. Data from 505 women with surgically/histologically confirmed endometriosis (WwE) and 505 control women (CW) matched for age were analysed. Women were recruited in eight Swiss and German hospitals between 2010 and 2015.
Participants/materials, setting, methods: Gynecologic and obstetric history, questions on quality of life, psychological wellbeing and socio-demographic data were collected using a self-administered questionnaire consisting of several validated questionnaires such as the Brief Pain Inventory, Perceived Stress Questionnaire 20, etc.
Main results and the role of chance: Data on miscarriages were provided by 954 women. There was no significant difference in the number of miscarriages between WwE (n = 74/of 470) and CW (n = 82/of 484). The distribution of the number miscarriages was similar in both groups: WwE: 54 with one, 13 with two, 7 with three and more miscarriages; CW: 57 with one, 18 with two and 7 with three and more miscarriages. In both groups the incidences of miscarriages increased with higher age. Staging for endometriosis was performed in 467/505 WwE. The number of women with miscarriages per WwE was not associated with the stage of the endometriosis: ASRM I: 18/87 (26%), II: 18/97 (18.5%), III: 20/132 (15.1%), IV: 17/151 11.2% (p = 0.21). The frequency of miscarriages was not correlated with specific localisations, such as ovarian (p = 0.218) or peritoneal (p = 0.094) endometriosis.

In contrast, among those women who wished to have a child, more women were childless in the group of WwE (52.5%) than in the CW group (13.1%) (p < 0.001).

WwE reported significantly higher emotional distress associated with undesired childlessness and reproductive therapy than CW (p = 0.001). They reported significantly more often feelings of inferiority (p = 0.005) but not feelings of depression (p = 0.17).
Limitations, reasons for caution: It was a retrospective, descriptive study conducted over several years at different centres. The endometriosis population, although very large, was heterogeneous for the duration of disease and the number of surgeries.
Wider implications of the findings: The abortion rate was not different in WwE, but childlessness and one-child families were significantly more frequent. As WwE experience significantly more emotional distress associated with childlessness and ART, and have more inferiority feelings than CW, they should be psychologically supported and counseled early about their reproductive potential and ART.
Trial registration number: ClinicalTrials.gov identifier: NCT02511626.

O-257 The oocyte and embryo quality are poorer in women with endometriosis compared to those without endometriosis

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Study question: Does endometriosis affect oocyte and embryo quality?
Summary answer: Women with endometriosis have lower number of oocyte collected per-mature follicle, unaffected fertilisation rate and higher rate of early embryo-arrest, which implicates poor oocyte quality.
What is known already: Endometriosis is known to be detrimental to fertility in many ways including poorer oocyte/embryo quality. Evidence drawn from studies on oocyte recipient cycles that showed poorer reproductive outcome in those who received oocytes from women with endometriosis, suggest that the oocyte quality of women with endometriosis is compromised. Studies that specifically examined the association between embryo quality and the presence of endometriosis or endometrioma are scarce and the question if endometriosis detrimentally impact on oocyte and/or embryo quality is still controversial.
Study design, size, duration: We performed a multi-centre retrospective evaluation of women with endometriosis undergoing IVF. We reviewed treatment cycle and embryology records via IDEAS™ database from January 2011 to December 2014. Data obtained from the database were exported to Excel (Microsoft, USA) before data analysis was performed using statistical analysis package. Comparative analysis was performed between groups and was expressed as means ± SD or percentages.
Participants/materials, setting, methods: Women who were <40 years old who underwent IVF treatment using their own gametes were included. The study group consisted of women who had endometriosis (EN) and the control group (tubal factor, TF and unexplained, UE) subfertility. Qualified and trained embryologists performed the oocytes and embryo assessment using Istanbul consensus workshop on embryo assessment by ESHRE special interest group of embryology.
Main results and the role of chance: Total of 678 women who had IVF treatment were analysed (EN, n = 89; TF, n = 214; UE, n = 375). The mean age for each group was EN, 34.4 ± 3.3; TF, 33.5 ± 3.7 and 34.6 ± 3.2 respectively. Number of mature follicles (>14 mm) on the day of trigger injection was similar (P = 0.746) between all groups. Percentage of oocytes collected per mature follicle was lower in endometriosis (EN, 65 ± 23; P = 0.008) compared to other groups (TF, 76 ± 20; UE, 71 ± 24). Fertilisation rate was similar across all groups (P = 0.758, EN, 67.5 ± 21; TF, 69.6 ± 21; UE, 69 ± 24, Higher percentages of embryos fail to achieve 8-cell stage in EN compared to TF groups [P = 0.02, MD 2.77, 95% CI (0.33, 5.21)] and UE [P = 0.03, MD 2.51, 95% CI (0.22, 4.79)]. Percentages of embryos at all grades (Grade 1–4) per women were similar between the comparison groups (P > 0.05). Endometriosis did not impair (P > 0.05) blastocyst development (P = 0.62, EN, 4.2 ± 2.5; TF, 3.8 ± 2.2; UE, 3.7 ± 2.2) or the development of Fully Expanded Hatching Blastocysts (FEHB) (P = 0.38, EN, 2.8 ± 2.1; TF, 3.0 ± 2.0; UE, 2.6 ± 1.9). The number of embryos utilised were similar among the comparison groups (P = 0.53, EN, 2.8 ± 2.1; TF, 2.9 ± 2.1; UE, 3.0 ± 2.2).
Limitations, reasons for caution: This study has following limitations: (1) restriction associated with a retrospective study, (2) subjective embryo assessment process despite using the newest assessment criteria, (3) incomplete assessment of the oocyte due to the intact cumulus cell layers required in IVF, and (4) unavailability of subgroups for the differential assessment of severity of disease.
Wider implications of the findings: The disparity between the number of collected oocytes and the number of mature follicles maybe due to the failure of oocytes to mature. The higher number of early embryo arrest at D1 suggests the presence of poor oocyte quality although further experimental studies will be required to confirm these findings.
Trial registration number: Nil.

O-258 ART outcomes in endometriosis-affected women after fresh versus frozen embryo transfer cycles: a matched cohort study

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Study question: To compare assisted-reproductive-technology (ART) outcomes between fresh versus differed frozen–thawed embryo transfer (dif-ET) in endometriosis women.
Summary answer: Frozen–thawed embryo transfer in endometriosis-affected women is associated with a significantly higher implantation rate, cumulative pregnancy rate and cumulative ongoing pregnancy rate.
What is known already: Controlled ovarian stimulation (COS) enhances the efficacy of ART by permitting multiple-oocyte yields, but also may alters endometrial receptivity by an advancement of endometrial development which could in turn contribute to diminished pregnancy chances. Recently, technical improvements in vitrification make Dif-ET a feasible alternative to fresh embryo transfer. In endometriosis-related infertility the eutopic endometrium is abnormal and its functional alterations are seen as likely to alter the quality of endometrial receptivity. One of the main questions in the endometriosis ART management is to know whether dif-ET could restore optimal receptivity in endometriosis-affected women leading to increase in pregnancy rates.
Study design, size, duration: This cohort study conducted in a tertiary care university hospital, evaluated all IVF/ICSI cycles for endometriosis infertility between 01/10/2012 and 31/12/2014. One hundred and thirty-five endometriosis women with a scheduled dif-ET cycle and 424 endometriosis women with a scheduled fresh ET cycle were eligible for matching. Diagnosis of endometriosis was based on published imaging criteria using transvaginal sonography and magnetic resonance imaging or histologically proven in women who had past surgery.
Participants/materials, setting, methods: Fresh and frozen–thawed ET were matched by age, number of previous ART cycles and endometriosis phenotypes (superficial peritoneal endometriosis, ovarian endometrioma or deeply infiltrating endometriosis). Two groups were compared: a group made up of "exposed" women (dif-ET group) who received only differed frozen–thawed embryos and an "unexposed" control group (fresh ET group), comprised of women who received fresh embryo(s) for the first transfer. Statistical analyses were conducted using univariate and multivariate logistic regression models.
Main results and the role of chance: A total of 270 women were included in the analysis: 135 in the fresh ET group and 135 in the dif-ET group. The cumulative clinical pregnancy rate was significantly increased in the dif-ET group compared to the fresh ET group [58 (43%) vs. 40 (29.6%), $p = 0.023$]. The implantation rate was 0.4 ± 0.5 in the dif-ET group and 0.2 ± 0.3 in the fresh ET group ($p < 0.001$). The cumulative ongoing pregnancy rate was 34.8% ($n = 24$) and 17.8% ($n = 24$) respectively ($p = 0.001$). After multivariate logistic regression, taking into account potential confounders as AMH level, type of protocol, total dose of injected gonadotropin (IU), type of embryo transfer, the number of oocytes retrieved and a previous history of endometrioma surgery the differed frozen–thawed embryo transfer was associated with a significant increase in cumulative ongoing pregnancy rate as compared to fresh ET (OR = 2.20, 95% CI 1.17–4.14, $p < 0.05$).
Limitations, reasons for caution: Given the significant higher rate of previous history of endometrioma surgery (and subsequent lower AMH levels) in Fresh ET group, we performed a logistic regression analyze to take into account such potential confounders. After multivariate analysis previous history of endometrioma surgery was not independently associated with IVF outcomes.
Wider implications of the findings: Our preliminary results evocate that differed frozen–thawed embryo transfer for endometriosis-affected women is an attractive option that could increase ART success rates. Future studies, with a randomized design, should be conducted to ascertain whether dif-ET enhances pregnancy rate in endometriosis-affected women.
Trial registration number: –

O-259 Serum AMH level is a misgiver in case of ovarian endometrioma

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Study question: Is there any correlation between serum anti-Müllerian hormone (AMH) levels and benign ovarian cyst size?
Summary answer: AMH serum levels are correlated with cyst size in case of OMA but not in case of non endometriotic cyst.
What is known already: Serum AMH level seems unrelated to the presence, dimension or nature of begin ovarian cyst but was found lower in women with bilateral benign ovarian cyst. In case of a previous history of OMA surgery, serum AMH levels are significantly reduced. Most previous studies have been conducted in infertile women and did not consider patient without past history of previous ovarian surgery. The strength of our study lies in the inclusion of women before first surgery.
Study design, size, duration: An observational, prospective, cross-sectional study of 228 non-pregnant women aged between 18 and 42 years and surgically explored for the first time for benign gynaecological disease, between January 2004 to June 2015, comparing AMH serum level according to presence and size of benign cyst. AMH levels were measured in serum samples drawn in the month preceding surgery.
Participants/materials, setting, methods: Women with available frozen serum sample for AMH dosage and without past history of gynaecological surgery were allocated in two group: women with histologically proven endometrioma (Study group, $n = 122$) and women with histologically proven benign ovarian cyst without endometriosis (Control group, $n = 106$).
Main results and the role of chance: AMH serum levels were not significantly different between the study and the control groups (4.0 ± 0.3 versus 3.9 ± 0.3 ng/ml; $p = 0.95$) although means age of women was significantly higher in the study group as compared to the control group (31.5 ± 0.4 and 29.1 ± 0.6; $p < 0.01$). (BB1) In the study group, serum AMH levels were higher in case of OMA diameter of more than 5 cm (3.4 ± 0.2 vs. 5.3 ± 0.5, $p < 0.001$). In addition we observed a significant positive correlation between the OMA diameter and AMH levels ($R^2 = 0.05$, $P = 0.009$) but not in the control group. Finally, in the study group no differences in serum AMH levels were found according to bilaterality or the existence of associated DIE lesions.
Limitations, reasons for caution: Our study population derived from a referral center for endometriosis surgery, included patients with severe forms of endometriosis requiring surgery. We cannot exclude that infertile women with OMAs associated with a diminished ovarian reserve were less likely to be referred for surgery and might therefore be underrepresented.
Wider implications of the findings: The link between AMH and OMA diameter may be responsible for an overestimation of ovarian reserve in such condition.
Trial registration number: None.

O-260 The role of environmental contaminants in the development of endometriosis: a systematic review

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[4]*Second University of Naples, Medical Statistics Unit, Naples, Italy*

Study question: What environmental contaminants could be involved in endometriosis?

Summary answer: Data suggest a relationship between endometriosis and several environmental pollutants. Specifically, organochlorines compounds represent the most suspected factors implicated in development of endometriosis.

What is known already: Based on experimental data, several pollutants have been implicated in the etiopathogenesis of endometriosis. However, more recent epidemiological studies have yielded contrasting results.

Study design, size, duration: A systematic review of the literature through electronic database was conducted without time restriction.

Participants/materials, setting, methods: Only articles and reviews on the association between endometriosis and environmental pollutants published in peer-reviewed journals were included. Exclusion criteria were: *in vitro* studies; Case reports or case series; unpublished data; studies involving animals; the use of not conservative statistic methods (e.g., Bayesian analysis). Studies with overlap between cases and controls and studies in which contaminants were not directly measured were also excluded.

Main results and the role of chance: The following articles were included in our analysis: 23 devoted to polychlorinated biphenyls (PCBs) and dioxin compounds; 10 to phthalate compounds; 5 to trace elements and heavy metals; 2 to organochlorine pesticides and bisphenols; and 1 to perfluorochemicals, particulate matter and benzophenone derivatives. Several studies indicated that dioxins and PCBs were involved in the etiopathogenesis of endometriosis. Specifically, the levels of polychlorinated dibenzo-p-dioxins (PCDDs), polychlorinated dibenzofurans (PCDFs) and PCBs (PCBs 138, 153, 180) were higher in patients with endometriosis than in control groups. There is also evidence that PCBs and dioxins play a role in the pathogenesis of deep endometriosis and adenomyosis. Results regarding the association between phthalates and endometriosis were contrasting. Regarding organochlorine pesticides, only β-hexachlorocyclohexane was associated with an increased odds of endometriosis. Only one study reported a significant association between perfluorochemicals (perfluorooctanoic acid and perfluorononanoic) and benzophenone metabolites (2,4-dihydroxybenzophenone) with endometriosis. The levels of such trace elements as copper and nickel were higher in endometriosis patients than in controls, however, a direct relation between trace elements and endometriosis has not been established. No association with bisphenols and particulate matter were reported in the few trials that addressed this issue.

Limitations, reasons for caution: Relevant differences were found among studies in terms of diagnostic method, type of pollutant analyzed, selection of controls and method adopted for the assessment of contaminates. Consequently, no definitive conclusion could be drawn regarding the association between pollutants and endometriosis.

Wider implications of the findings: Based on currently available data, an association between endometriosis and environmental pollution cannot be excluded. More prospective well-designed and less heterogeneous trials are warranted to shed light on this issue.

Trial registration number: N.A.

INVITED SESSION

SESSION 66: BASIC SCIENCE OF EARLY PREGNANCY

Wednesday 06 July 2016 **Hall 1** **12:00–13:00**

O-261 Molecular regulation of implantation

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Implantation failure remains a major problem after embryo replacement, and experimental approaches to discover molecular mechanisms are required so that potential therapeutic approaches can be tested. We transferred blastocysts developing from 84 human fresh or frozen embryos to a constitutively receptive epithelial (Ishikawa) cell monolayer. Chemical hatching of blastocysts was adopted in the course of the experimental series in order to minimise variation in attachment kinetics. We observed stable attachment of all hatched embryos, progressing in some cases to breaching of the epithelium. Evidence of limited local apoptosis of epithelial cells was gained by expression of activated caspase 3 and nuclear fragmentation. Blastocoel collapse was usually observed. After 48 h of coculture (day 8), trophoblast differentiation had already begun and breaching of the epithelium was observed at multiple independent sites, with primary syncytium always involved. CDX2 and GATA3 were expressed by distinct subpopulations of cytotrophoblast, respectively proximal and distal to the blastocoel cavity. As expected, expression of HLAG and CG was initiated. Osteopontin, an epithelial secretory glycoprotein with pro-adhesive properties, and its receptors CD44 and integrin αvβ3, were all present at the human attachment sites.

Hatched mouse blastocysts also attach to the human epithelial monolayers, but require activation in order to progress to displacement and invasion. Though we have evidence that endogenous osteopontin and its receptors are involved in attachment, addition of exogenous recombinant human osteopontin did not advance implantation extent or speed.

Data obtained with both species of embryo suggest that the interaction between trophoblast and endometrial epithelium initiates differentiation of trophoblast, including stimulating the formation of multiple foci of primary syncytium and differentiated mononuclear cytotrophoblasts. Thus the early interaction with epithelium may have important influences on later stages of the implantation cascade.

O-262 H-Y immunity in early pregnancy

H. Svarre Nielsen[1]

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Background: Approximately half recurrent pregnancy loss (RPL) cases remain unexplained after standard investigations. Secondary RPL (SRPL) is, in contrast to primary RPL, preceded by a birth, which increases the transfer of fetal cells into the maternal circulation. In previous RCT of immune therapy for SRPL we noted that eligible SRPL women more frequently had given birth to boys prior to the pregnancy losses than a girl. Mothers of boys are often immunized against male-specific minor histocompatibility (H-Y) antigens. H-Y immunity can cause graft-versus-host disease after stem-cell transplantation. We proposed the *H-Y hypothesis* that aberrant H-Y immunity is a causal factor for SRPL.

Methods: Epidemiological, immunogenetic and immunological studies in a 20 year cohort of unexplained SRPL (n = 358 women). Population and registry based epidemiological studies on the impact of previously born boys on subsequent obstetric outcomes.

Results: SRPL is more common after the birth of a boy and the subsequent live birth rate is reduced for SRPL patients with a firstborn boy. The male:female ratio of children born prior and subsequent to SRPL is 1.49 and 0.76 respectively. Maternal carriage of HLA-class II alleles presenting H-Y antigens to immune cells is associated with a reduced live birth rate and increased risk of obstetric complications in surviving pregnancies in SRPL patients with a firstborn boy. In early pregnancy, both antibodies against HLA and H-Y antigens are increased in SRPL patients compared with controls. Presence of these antibodies in early pregnancy is associated with a lower live birth rate and a low male:female ratio in subsequent live births, respectively. Births of boys are also associated with preeclampsia/eclampsia, placental abruption, stillbirth and preterm birth in subsequent births in the background population.

Conclusions: Epidemiological, immunogenetic and immunological studies support the hypothesis that aberrant maternal H-Y immune responses have a pathogenic role in SRPL.

INVITED SESSION

SESSION 67: ASRM/SOCIETY FOR REPRODUCTIVE ENDOCRINOLOGY AND INFERTILITY: PRIMARY OVARIAN INSUFFICIENCY: THE PROBLEM AND POSSIBLE SOLUTIONS

Wednesday 06 July 2016 **Hall 5 CB** **12:00–13:00**

O-263 The new science of folliculogenesis and follicle depletion: fact and fiction

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Oogenesis in women is inextricably linked and dependent upon proper folliculogenesis. Following migration of primary germ cells to the gonadal ridge and expansion of the germ cells, primordial follicles are formed with investment of oocytes by surrounding cells. Later in development these cells will nurture and support oocyte maturation. The primordial follicles may remain dormant for decades before resuming growth and development, but the brake holding the follicles in check is enigmatic and may be considered an unknown "X" factor. Knowledge of the processes involved in maintenance of follicle quiescence is crucial to an understanding ovarian aging and premature ovarian aging (diminished ovarian reserve) and has relevance to fertility preservation. Genetic and epigenetic approaches have emphasized the importance of telomere length and some key genes for oocyte maintenance; but although important, genetic mechanisms do not fully explain follicular quiescence. While the mechanisms that maintain follicle and oocyte quiescence remain incompletely defined, recent research has provided insight into some key signaling pathways involved. Still, much remains unknown and considerable confusion remains. For instance, conflicting reports exist regarding ovarian stem cells and the derivation of eggs from ovarian stem cells. Additionally, key questions remain unanswered, such as: what processes are critical to the storage of an oocyte for decades? Increasingly, work in several laboratories throughout the world have pointed to the crucial role of biomechanical signaling and mechanotransduction in oocyte maturation and folliculogenesis. Collectively, pioneering work in the laboratories of Drs. Evans, Woodruff, Hsueh, Conti, He and Kawamura have provided exciting insight into oocyte and follicle maturation. Importantly, these recent reports in murine and human species have suggested that mechanical signaling may be exploited to activate dormant follicles. Moreover, and taken together, the reports suggest that mechanical signaling itself may represent a key component to the "X" factor involved in maintenance of follicle quiescence. This presentation will examine the recent research on oogenesis and folliculogenesis in women. The objective is to answer key questions, to summarize recent developments with a focus on mechanical signaling, and to suggest new directions for future research.

O-264 Fertility treatment for women with POI-where are we are and where are we going?

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The rate of natural conception after a diagnosis of Primary Ovarian Insufficiency (POI) is commonly quoted to be in the range of 5–10%. However, this rate may be an overestimate, particularly for women with an advanced stage of POI presenting at an older age than in the past. With early diagnosis of diminishing ovarian reserve, natural conception or cryopreservation of oocytes may be possible. Oocyte cryopreservation is now commonly offered to women about to undergo gonadotoxic therapy. Early identification and appropriate counseling are needed to maximize the chance that a woman at risk of POI will be able to conceive with autologous oocytes.

Intermittent ovarian follicular development may occur with POI, but follicles may by dysfunctional and contain atretic oocytes. Ovulation induction for women with POI has been attempted with strategies such as administration of corticosteroids or suppression of endogenous gonadotropin (with gonadotropin releasing hormone agonist or estrogen and/or progestin) followed by ovarian stimulation with exogenous gonadotropin. It has been suggested that normalization of the elevated serum luteinizing hormone level common with POI could potentially prevent luteinization of ovarian follicles, and thereby improve follicle function. Conception is possible for some women with POI, even with undetectable anti-Müllerian hormone levels. However, for women with POI diagnosed at an advanced stage, oocyte or embryo donation are the only evidence-based treatments. *In vitro* maturation of primordial follicles as well as creation of induced pluripotent stem cells (iPSCs) are options being explored. Live births have been reported with an experimental treatment, *in vitro* activation, which may prove to be helpful for some women with POI.

SELECTED ORAL COMMUNICATIONS

SESSION 68: EMBRYO DEVELOPMENT AND NON-INVASIVE ASSESSMENT

Wednesday 06 July 2016 **Hall 1** **14:00–15:15**

O-265 Follicular fluid biomarkers for human embryo quality *in vitro* fertilization: proof of principle

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Study question: Is the peptide profile in human follicular fluid (FF) associated with embryo quality in IVF/ICSI and can we use it to identify potential?
Summary answer: We demonstrated a panel of 24 peptides that were differentially detected in FF corresponding to embryos of top quality and poor quality.
What is known already: Human follicular fluid (FF) is a unique biological fluid in which the oocyte develops *in vivo*, and presents an optimal source for non-invasive biochemical predictors. A number of single peptides or proteins have already been assayed in FF and some seem to correlate with oocyte maturation. But most of the studies focused on known proteins, and rarely correlated them to embryo quality. Given the complexity of the numerous independent processes involved, it is unlikely that a single biomarker can predict embryo quality. Application of powerful peptidomic technologies may significantly contribute to biomarker discovery and also better understanding the reproductive processes.
Study design, size, duration: A total number of 67 individual follicular fluid samples from couples undergoing IVF/ICSI treatment at the Leuven University Fertility Center were analyzed in discovery and replication cohorts separately. All the patients were younger than 36 years old and underwent the first or second cycle. Samples were collected from January 2013 to September 2015.
Participants/materials, setting, methods: Follicular fluid with good embryos and non-good quality embryos were split into discovery (n = 51) and replication (16) cohorts. A total of 23 (45%) participants from the discovery cohort and 6 (37.5%) participants from the replication cohort were responded to poor quality embryos. The discovery cohort were split into two experiments, with 15 (8/7) and 36 (20/16) samples, respectively. Peptide profiles were acquired by nano-scale liquid chromatography coupled to tandem mass spectrometry (nano LC-MS/MS).
Main results and the role of chance: Twenty-four peptides were retained as potential biomarker candidates, distinguishing 45 out of 51 embryos in discovery experiment. The 24 peptides panel was further validated in the replication cohort and demonstrated an accuracy of 81.2% (13/16). A total number of 106 proteins were validated, reporting a protein false discovery rate (FDR) of 0.00% and peptide FDR of 0.00%. Seven potential biomarker peptides could be identified. They are involved in immune responses, complement activation, extracellular matrix binding, ion channel binding and cell adhesion.
Limitations, reasons for caution: The different methodologies and analytical tools each bring their own selectivity. The challenge of peptide concentration variability across samples and experiments remains a major constraint.

Wider implications of the findings: This study, for the first time, presents a panel of peptides in FF can be used as potential biomarker to predict quality of embryos. It also reveals that peptide profiling in individual follicular fluid samples can be a new promising innovative, non-invasive approach to assist embryologist with embryo selection.
Trial registration number: None.

O-266 Prediction of embryos at risk of chromosomal abnormalities and developmental arrest by metabolic profiling of >100 metabolites in spent culture media

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[5]*Aristotle University, Laboratory of Histology and Embryology, Thessaloniki, Greece*
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Study question: Can metabolic profiling predict embryos at risk of chromosomal abnormalities and developmental arrest?
Summary answer: Metabolic profiling can predict aneuploid and chaotic embryos that arrest in culture and normal embryos that result in a viable pregnancy.
What is known already: Non-invasive methods of embryo selection including cell free mt-DNA and metabolic profiling have been proposed to distinguish between embryos with the potential to implant and those that arrest. Down's syndrome embryos and Monosomy 21 embryos have previously been shown to have differential expression of metabolites compared to normal embryos, while limited studies, have investigated in detail the metabolic profiling of embryos with other abnormalities in comparison to chromosomally normal embryos.
Study design, size, duration: This an ongoing study, initiated in 2011. 375 embryos cultured in 4 different culture media (Irvine $n = 80$, Origio $n = 90$, Sage $n = 138$, Vitrolife $n = 67$) were subjected to day 3 biopsy and processed for PGD/S. Culture media prior to biopsy (day 1–3) and following biopsy (day 3–5) were collected and analysed by hydrophilic interaction liquid chromatography tandem mass spectrometry HILIC-MS/MS.
Participants/materials, setting, methods: PGD/S was conducted in a private IVF Unit and an academic hospital with IVF/PGD laboratory. Metabolic analysis was conducted in a Forensic Toxicology Laboratory by HILIC-MS/MS which was developed to provide the quantitation of circa 100 metabolites in an UPLC system with a triple quadrupole spectrometer.
Main results and the role of chance: This study provides, so far, the largest series screening for >100 primary metabolites using HILIC-MS/MS in 4 different spent culture media (Irvine, Origio, Sage, Vitrolife). UPLC conditions were optimized for each culture medium separately and reached maximum peak capacity and retention for all hydrophilic metabolites in a single run of 40 min. The methodology was validated and proved to be reliable and robust. Unique biomarkers, in spent culture media, from embryos diagnosed as abnormal following PGS and reconfirmed as uniformy aneuploid, major-mosaics or chaotics following meta-analysis of all the nuclei on day 5, were identified and characteristic patient specific metabolic profiles were observed, which differed between chromosomally normal embryos that had developed to the blastocyst stage and resulted in a viable pregnancy and aneuploid and chaotic embryos that arrested in culture. Logistic regression analysis revealed a number of metabolites that had a high predictive value and models were created which in the future could serve as non-invasive markers for the detection of chromosomal abnormalities before embryo transfer.
Limitations, reasons for caution: Although all abnormal embryos were meta analysed on day 5 to confirm initial single cell diagnosis and their uniform or mosaic status was established, normal embryos transferred on the basis of single cell analysis but not resulting in a pregnancy could not obviously be tested for mosaicism.
Wider implications of the findings: This study provides unique biomarkers found in 4 different spent media from chromosomally abnormal embryos. Comparison of these metabolic profiles with those of chromosomally normal embryos by logistic regression showed distinct differences, which in the future could serve as non-invasive markers for the detection of chromosomal abnormalities before embryo transfer.
Trial registration number: A9850/11.

O-267 Detecting metabolic differences in mouse and human embryos through a novel imaging strategy

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[4]*Harvard, Stem Cell and Regenerative Biology, Cambridge, MA, USA*

Study question: Are there distinct metabolic changes that occur in embryonic development that can be detected with metabolic imaging, and can they be used to select the healthiest embryos?
Summary answer: Metabolic imaging can sensitively detect shifts in embryo metabolism over the course of embryo development, and can distinguish between embryos of different metabolic states.
What is known already: Metabolic function changes as embryos develop, with both varying degrees of ATP synthesis and turnover at different developmental stages. Ratiologic redox fluometry was developed as a means of metabolic imaging using single photon autofluorescence of FAD and NAD, which are both integral components in the production of ATP. Although this form of imaging has been studied in human systems including brain tissue, cardiac and cancer cells, it has not yet been applied to embryos. This technology poses an advantage to current non-invasive embryo assessment tools including time lapse imaging and indirect assessment methods of embryo metabolism such as metabolomics.
Study design, size, duration: Mouse embryos were obtained from a commercial source. 100 embryos were used in this cohort study, while the metabolic imaging scope was optimized for embryo monitoring over a 1-year duration. The on-stage incubation system was used for long-term monitoring of cellular metabolism over the course of embryo development from the 1-cell to blastocyst stage. Human embryos selected for imaging were donated under an IRB approved protocol.
Participants/materials, setting, methods: Metabolic measurements were obtained by measuring NADH and FAD autofluorescence using fluorescence lifetime imaging microscopy (FLIM). Images were obtained every 3 h during the course of mouse embryo development using an on stage incubation system. Human embryos were thawed using a commercial thaw kit and metabolic images were acquired several hours after thawing. Embryos were then cultured in a conventional incubator to the day 5 Stage of development to assess for continued development after imaging.
Main results and the role of chance: FLIM-based metabolic imaging was able to distinguish between metabolic states in three separate experiments. First, cohorts of oocytes from old (9-month) vs. young (<3-month) mice were measured, displaying distinct metabolic profiles. As oocyte metabolic health and viability are known to decline with maternal age, this highlights the likely predictive potential of this imaging technology. Second, we observed distinct changes in measured metabolic FLIM signatures over the course of mouse embryo development from the 1-cell to the blastocyst stage.

In particular, NADH abundance peaks around compaction and decreases significantly during blastocyst formation, likely corresponding with an associated increase in energy demands. This pattern was preserved among the embryo cohort, and was significant with respect to measurement error. Through these experiments, we obtained real-time, high time-resolution, yet non-invasive representations of metabolic development. The continued development of the mouse embryos was consistent with controls and expected outcomes from the commercial distributor, despite many image captures throughout the course of development.

Finally, images of discarded human embryos were acquired at a single point in development. These embryos showed various aberrant morphologies. Correspondingly to the different forms of developmental failure, each embryo's metabolic measurement was significantly distinct from the others, indicating different metabolic states.

Limitations, reasons for caution: Although these findings represent an innovative mechanism of embryo evaluation, there is no defined standard for interpreting metabolic FLIM parameters. The hardiness of the mouse model presents a challenge to testing the implications of this method on mouse embryo development. Human embryo measurements represent only a preliminary data set.

Wider implications of the findings: This work represents a foundational tool for assessing the metabolic health of embryos. Preliminary results indicate promise for the use of metabolic imaging as a potential embryo selection tool. Future in-clinic measurements on clinical samples are planned, and will investigate possible correlations between metabolic signatures and final pregnancy outcomes.

Trial registration number: N/A.

O-268 Cell allocation patterning between the two-cell to the blastocyst stage might be associated with organ development in mouse offspring

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Study question: Does cell allocation patterns during pre-implantation development affect implantation and further embryo development?

Summary answer: Cell allocation patterns from the two-cell stage embryo up to the blastocyst stage affect further embryo development and offspring heart weight in mice.

What is known already: During pre-implantation embryo development, the first cell fate decision is observed at blastocyst stage. The inner cell mass is situated at the embryonic (Em) axis and the trophectoderm at the abembryonic (Ab) axis. Some authors claim that the Em-Ab axis can be predicted by the first embryo cleavage plane which is orthogonal to the Em-Ab axis in the majority of embryos. However, this theory has been debated thoroughly in the literature. Also, the correlation between the first embryo cleavage plane with the Em-Ab axis and further putative offspring development has been demonstrated only in cloned mouse embryos.

Study design, size, duration: One-cell frozen B6C3F1 × B6D2F1 mouse embryos (n = 568, 3 repetitions) were thawed and cultured; from those, 259 classified blastocysts according to their cell allocation pattern were transferred to 2.5 days post-coitus (dpc) pseudo-pregnant CD-1 females (n = 17) by non-surgical embryo transfer (NSET) method. Two weeks old live offspring (n = 45) were dissected and heart, spleen, lungs, liver, kidneys were weighed. Pregnancy and implantation rates were also recorded.

Participants/materials, setting, methods: Embryos had one blastomere injected at the two-cell stage with a lipophilic tracer and *in vitro* cultured to the blastocyst stage. Then, blastocysts were classified into three groups: orthogonal, if the Em-Ab axis was orthogonal to the borderline between labelled and non-labelled cells; deviant, if it was parallel; or random, if labelled and non-labelled cells were intermingled. NSET was used to transfer classified blastocysts into pseudo-pregnant females. Progeny were sacrificed on day 14 for organ morphometry.

Main results and the role of chance: From the total cohort of blastocysts studied, those classified as random were significantly higher (52.3%, P = 0.037); when compared with orthogonal (27.0%) and deviant (28.3%) embryos. No significant differences were found among the three groups in terms of pregnancy rates (66% orthogonal, 83% deviant and 100% random), implantation rates (42% orthogonal, 39% deviant and 50% random), pregnancy loss (49% orthogonal, 79% deviant and 55% random), offspring total body weight (11.1 ± 0.4 g orthogonal, 9.7 ± 0.4 g deviant and 10.8 ± 0.2 g random) and relative weight (organ weight/total weight) of lungs, liver, spleen and kidneys (P > 0.05). However, a significant difference was found in the heart weight between the three groups (P = 0.023). The deviant blastocyst group resulted in offspring with the smaller heart weight.

Limitations, reasons for caution: The mechanism(s) causing the cell allocation patterns during pre-implantation development are not understood. The sample size was small and other effects would have reached statistical significance with more repeats. Patterning in human embryos has not been studied and so it is difficult to determine the clinical translation of these findings.

Wider implications of the findings: There is a defined presence of three different embryos according to their cell allocation pattern; correlation of these patterns with implantation and further development must be established (if any). If so, new embryo selection strategies might stem from this work to improve success rates after assisted reproductive treatments.

Trial registration number: N/A.

O-269 Inner cell mass of blastocyst grade and loosening of inner cell mass is related to monochorionic diamniotic twinning

J. Otsuki[1], T. Iwasaki[1], Y. Katada[1], H. Sato[1], Y. Tsutsumi[1], K. Hatano[1], Y. Tsuji[1], K. Furuhashi[1], Y. Matsumoto[1], S. Kokeguchi[1], M. Shiotani[1]
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Study question: Is inner cell mass (ICM) grade and morphological configuration is related to the occurrence of monochorionic diamniotic (M-D) twinning?

Summary answer: Yes, low ICM grade and loosening of ICM can be the risk factors of M-D twinning.

What is known already: Monozygotic twinning (MZT), which includes monochorionic diamniotic (M-D), monochorionic monoamniotic (M-M) and monozygotic dichorionic diamniotic (D-D) is known to increase with ART procedures. Extended culture, younger age, oocyte/embryo manipulations such as assisted hatching (AH) and ICSI have been considered to contribute MZT, however, they are still controversial and it remains unknown what factors contribute to that increased risk.

Study design, size, duration: Retrospective cohort study involving 8435 women who underwent frozen–thawed single blastocyst transfer with hormone replacement treatment (HRT) between January 2011 and December 2014. 71 ICM of blastocysts observed by time lapse system (Embryo Scope) from June 2013 to December 2014 were also retrospectively analyzed.

Participants/materials, setting, methods: Statistical analyses were carried out to explore the cause of MZT. Any changes in configuration of the ICM observed by time lapse system was retrospectively analyzed. Loosening of the ICM was defined as when more than 5 cells were loosened after a tight ICM was once observed by the time lapse system. Pregnant patients underwent a vaginal ultrasound and monozygotic twinning (M-D, M-M and D-D) was confirmed at both 6–7 and 8–9 weeks.

Main results and the role of chance: A total of 8435 frozen–thawed single blastocyst transfer with HRT resulted in 3445 (40.8%) clinical pregnancies having a total of 80 MZT (2.32%). The number of M-D, M-M D-D twins and monochorionic triplet were 36 (1.04%), 3 (0.09%), 39 (1.13%) and 2 (0.06%) respectively. Gender discordance in twins was found in two D-D cases. The rate of dual live birth was also significantly higher in M-D twinning than in D-D twinning (p = 0.0017). The M-D twinning rate when blastocysts with a high grade ICM (grade A) were transferred was 0.38% (3/796), whereas it was 1.38% (34/2463) when blastocysts with a lower grade ICM (grade B and C) were transferred, and there was a significant difference between the two groups (p = 0.033). 71 frozen–thawed blastocysts were transferred after observation by time-lapse system. The hCG, GC, FHB positive rates were 74.2% (52/71), 63.4% (45/71), 57.7% (41/71) respectively, and there were two D-D and one M-D twins. Among the 41 cycles with positive FHB, 36 cases were delivered, 4 cases miscarried and 1 case lost contact. By careful observation of the embryo resulting in the one M-D case, it was found that the ICM became loose as a result of having 8 cells de-compacted, which was not observed in the other ICM of transferred blastocysts.

Limitations, reasons for caution: The birth of babies derived from natural conception cannot be eliminated although the occurrence is very small. As the occurrence of M-D twinning is around 1% and availability of data and time-lapse systems are limited, multi-center study is necessary to confirm these findings.

Wider implications of the findings: The occurrence of M-D twinning can be reduced by avoiding the transfer of embryos when the loosening of ICM is confirmed by time-lapse observation in addition to observing splitting of two or three inner cell masses in an expanded blastocyst.
Trial registration number: Not applicable.

INVITED SESSION

SESSION 69: ENDOMETRIOSIS, HOW DOES IT HURT?

Wednesday 06 July 2016 **Hall 5 A** **12:00–13:00**

O-270 Peripheral nerve mechanisms in endometriosis associated pain

M. Morotti[1]
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Pain remains the cardinal symptom of endometriosis. Similar to many other chronic conditions pain sensation in endometriosis appears to be a close interaction between the nociceptors and peripheral nerve fibres and central and hormonal modulation.

However, the exact pathomechanisms are still only poorly understood. This presentation focuses on factors important in the peripheral pain system.

Endometriotic lesions consist of glandular epithelial and stromal cells. Currently, endometriotic tissue is divided into three different entities: (a) peritoneal lesions, (b) ovarian cysts and (c) deep endometriosis. None of these locations/types of disease are correlated with a specific pain quality or severity.

Recently it was demonstrated that nerve fibres are found in close proximity to endometriotic lesions.

Mounting evidence indicates that these fibres are recruited in parallel to blood vessels through a process termed neuroangiogenesis. Blood vessel and nerve growth are linked by common pathways that involve the release of pro-angiogenic factors, such as vascular endothelial growth factor, β-nerve growth factor and neuropeptides.

Proangiogenic factors are also known to stimulate nerve growth, and molecules produced by vascular cells could both stimulate and guide nerve growth.

These factors are produced via different processes, not least by immune cells that are ubiquitously found in the peritoneal cavity of women affected by the condition. Conversely, the peripheral nerve fibres take an active part in inflammatory processes by regulating effector cell function and reallocation of energy to the immune system.

The upregulation of neurotrophic and angiogenic factors in peritoneal fluid of women with endometriosis, the increase of new nerve fibres and their spatial vicinity with the lesions have led to increased interest in peripheral changes in endometriosis-associated inflammation and pain generation.

The main features of this peripheral process are loss of sympathetic nerve fibres from sites of inflammation concomitant with increased innervation with sensory nerve fibres and increased sensory nerve fibre activity.

However, a clear link between the amount of new nerve fibres and pain severity in patients with endometriosis has so far not been demonstrated.

O-271 Central pain mechanisms relevant to endometriosis-associated pain

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It is well known that the severity of pain experienced by women with endometriosis correlates poorly with the extent of disease identified at laparoscopy. This observation suggests that factors other than the ectopic lesions are involved in the generation and/or amplification of pain. The experience of pain is both sensory and emotional and involves coordinated activity within a distributed network of brain regions. The brains of patients with chronic pain exhibit both structural and functional differences when compared to pain-free controls. Moreover, these changes are remarkably similar across chronic pain conditions, no matter what the underlying pathology. This presentation will firstly summarise the central changes associated with chronic pain and consider how they may apply to women with endometriosis-associated pain. Recent findings from functional brain imaging studies of women with endometriosis-associated pain will then be described and the potential of these findings to inform our clinical practice explored.

SELECTED ORAL COMMUNICATIONS

SESSION 70: SCREENING AND PREDICTING

Wednesday 06 July 2016 **Hall 5 CB** **14:00–15:15**

O-272 Implementation of an expanded carrier screening test for recessive genetic disorders in an oocyte donation program

A. Abuli Vidal[1], M. Boada[1], E. Clua[1], L. Latre[1], M. Luna[1], B. Rodríguez-Santiago[2], B. Coroleu[1], L. Armengol[2], L. Perez-Jurado[3], A. Veiga[1], X. Estivill[1], B. Pedro N[1]
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[3]*Pompeu Fabra University, Genetics Unit, Department of Experimental and Health Sciences, Barcelona, Spain*

Study question: Next-generation sequencing (NGS) is revolutionizing genetic research and diagnosis in reproductive medicine. What can expanded carrier screening (ECS) contribute to in an oocyte donation (OD) program?
Summary answer: The emergence of NGS-technologies has allowed the implementation of a NGS-based screening in an OD program to reduce the rate of livebirth with genetic disease.
What is known already: Genetic screening of gamete donors (GD) consists mainly of the assessment of personal medical and family history by a geneticist. However, many specific inherited conditions can remain unnoticed in a family. Therefore, based on scientific societies' recommendations, most ART centres include the karyotype and targeted-mutation screening for cystic fibrosis and Fragile X syndrome. In recent years, the cost of molecular genetic studies has been reduced due to the emergence of NGS-technologies, allowing the analysis of a large number of genes in a cost-effective manner. This claims for the extension of carrier screening to a larger number of genetic conditions.
Study design, size, duration: We describe the clinical implementation of a NGS-based panel (qCarrier test) for expanded carrier screening (ECS) in an OD program. The qCarrier test includes 200 genes associated with 218 autosomal recessive and 22 X-linked diseases. Our protocol in the OD program includes the karyotype and the qCarrier test. ECS is performed to OD candidates and the male partner of oocyte recipients. All patients have a pre and post-test genetic counselling session.
Participants/materials, setting, methods: The studied population consists of 1,118 individuals (483 oocyte donor candidates and 635 male partners of oocyte recipients) evaluated in the framework of an OD program. Carriers of X-linked conditions were discarded from the OD program, while a carrier state for an autosomal recessive condition was not an exclusion criterion for the female donors. We performed a manual "genetic-match" so that the donor and the recipient were not carriers for the same recessive condition.
Main results and the role of chance: The clinical implementation of ECS in our OD program has been in approximately 86% (545/635) of gamete donation cycles. We detected 1,102 pathogenic or likely-pathogenic mutations in the analyzed genes. The 10 most common autosomal recessive conditions detected with qCarrier test include: non-syndromic deafness, cystic fibrosis, galactosemia, phenylketonuria, familial Mediterranean fever, spinal muscle atrophy, alpha thalassemia, Smith–Lemli–Opitz syndrome, polycystic kidney disease and classical congenital adrenal hyperplasia. We identified 56.3% subjects who were carriers of at least one mutation in a gene responsible for a Mendelian genetic disease. The majority (64.1%) were carriers of only one mutation. The average carrier burden of genetic conditions was of 0.56 mutations per sample. In the context of our OD program, approximately 1.7%

(8/483) of oocyte donors were discarded because they were carriers of an X-linked condition and, we identified 3% (19/635) of pre-assigned donor–recipient matches with high reproductive risk for an autosomal recessive disease, which required a change in the selection performed. With the use of an ECS test we have decreased by 0.75% the risk of births with a genetic condition. Genetic counselling is an essential step before and after performing the genetic screening.
Limitations, reasons for caution: The limitations are the identification of previously unknown variations that challenge the communication of results and the psychological impact of the ECS results in oocyte donors. There is an absolute need for an adequate pre and post-test genetic counselling that clearly states the advantages, limitations and implications of the test.
Wider implications of the findings: We plan to offer the ECS to couples with reproductive projects, since they might also benefit from knowing their carrier status. Regarding technical improvements, it would be relevant to analyze the complete coding sequence of genes, expand the targeted genes to additional disorders and measure nucleotide-repeat expansions from NGS data.
Trial registration number: No clinical trial.

O-273 Repeated predictions of natural conception for the same couple

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Study question: How can we predict chances of natural conception in the same couple at various time points?
Summary answer: We developed a dynamic prediction model that can make repeated predictions over time for the same couple.
What is known already: The most frequently used prediction model for natural conception (the "Hunault model") is able to estimate the probability of a natural conception only once per couple, that is, after the completion of the fertility workup. This model cannot be used for a second or third time in the same couple who wish to know their renewed chances after a certain period of expectant management.
Study design, size, duration: A prospective cohort studying the long term follow up included subfertile couples in 38 centres in The Netherlands between January 2002 and February 2004. Couples with bilateral tubal occlusion, anovulation, or a total motile sperm count $<1 \times 10^6$ were excluded.
Participants/materials, setting, methods: The primary endpoint was time to a natural conception, leading to an ongoing pregnancy. Follow up time was censored at the start of treatment or at the last date of contact. We included the same predictors in our dynamic prediction model as the Hunault model, i.e., female age, duration of subfertility, female subfertility being primary or secondary, sperm motility and referral status. The performance of the model was evaluated in terms of calibration and discrimination.
Main results and the role of chance: Of the 4,999 couples in the cohort, 1,053 (21%) women reached a natural conception leading to an ongoing pregnancy within a mean follow up of 8 months (range 1–66 months). According to our newly developed dynamic prediction model, the median predicted probability in the first year after completion of the fertility workup was 27%. If pregnancy did not occur in the first half year, the median predicted chance of conceiving in the next year was 20% and decreased to 15% and 13% after 1 year and after one and a half years of unsuccessful expectant management, respectively. The model appeared to perform fair up to two and a half years of follow up in an internal validation.
Limitations, reasons for caution: The dynamic prediction model needs to be validated in an external population.
Wider implications of the findings: This dynamic prediction model allows reassessment of natural conception chances after various periods of unsuccessful expectant management. This gives valuable information to counsel couples with unexplained subfertility.
Trial registration number: Not applicable.

O-274 Biomarkers of ovarian reserve as predictors of reproductive potential

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Study question: Are biomarkers of ovarian reserve: anti-Müllerian hormone (AMH), inhibin B, and follicle stimulating hormone (FSH) predictive of reproductive potential, as measured by fecundability, in an older, reproductive age cohort?
Summary answer: Biomarkers of ovarian reserve are not predictive of reproductive potential in an older, reproductive-age cohort.
What is known already: Biomarkers of ovarian reserve, including AMH and early follicular phase inhibin B and FSH levels, have been shown to predict time to menopause and response to ovarian stimulation following controlled ovarian hyperstimulation. There is some evidence that they predict pregnancy after assisted reproductive technology. Prior studies on their ability to predict natural fertility have been conflicting. Urinary FSH kits are being marketed to women as tests for future reproductive potential.
Study design, size, duration: 755 women were enrolled in a prospective time-to-pregnancy cohort study, designed to determine the predictive value of biomarkers of ovarian reserve. Women provided a serum and urine sample at enrollment on menstrual cycle day 2, 3, or 4. They were followed until pregnancy was detected or up to 12 months of attempt.
Participants/materials, setting, methods: Women, 30–44 years old, with no history of infertility, who were trying to conceive for less than 3 months, were recruited from the community. Serum was analyzed for FSH, inhibin, and AMH. Urine was analyzed for FSH and creatinine-corrected (uFSHcr). uFSHcr was dichotomized at a cut-point equivalent to a serum value of 10 mIU/ml. Discrete Cox models were used to calculate fecundability ratios (FR) adjusting for age, race, prior pregnancy, body mass index, and smoking history.
Main results and the role of chance: Median AMH values (interquartile range) were 2.77 ng/ml (1.44–5.26) and FSH 6.6 mIU/ml (5.26–8.19). Ten percent of women had a urinary FSH value greater than 11.5 mIU/mg cr. AMH and age were inversely correlated ($r = -0.28$, $p < 0.001$). uFSHcr levels were correlated with serum FSH levels ($r = 0.62$, $p < 0.001$). AMH was not associated with fecundability [FR: 0.97, 95% Confidence Interval (CI): 0.89–1.06]. Neither was serum FSH (FR 0.92, 95% CI: 0.73–1.14) nor inhibin b (FR 1.0, 95% CI: 0.99–1.01). No significant association was observed between uFSHcr and fecundability when modeled as a continuous variable (FR: 1.14, 95% CI: 0.97–1.34) or dichotomized (FR: 0.99, 95% CI: 0.71–1.39). Limiting the sample to older women (≥35 years) had little impact (AMH: FR 1.03, 95% CI: 0.88–1.20; FSH: FR 0.92, 95% CI 0.59–1.44).
Limitations, reasons for caution: Pregnancy, not live birth, was the outcome for this study.
Wider implications of the findings: In this large, time-to-pregnancy study of women at risk for ovarian aging, we did not observe an association between markers of ovarian reserve and fecundability. Biomarkers of ovarian reserve do

not appear to predict reproductive potential independent of age. Women should be discouraged from using them as fertility tests.
Trial registration number: NCT01028365.

O-275 Oocyte cytoplasmic maturation, metaphase plate alignment and aneuploidies – impact on fertilization rate, embryo quality and pregnancy outcomes

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Study question: Is there a relationship between the oocyte cytoplasmic maturation and the metaphase plate conformation and aneuploidies?
Summary answer: An incomplete oocyte cytoplasmic maturation is related to metaphase plate anomalies and aneuploidies. Outcomes were poorer in cycles with oocytes from cohort with cytoplasmic immaturity.
What is known already: Nuclear and cytoplasmic oocyte maturation occurs *in vivo* during follicular growth and ovulation. Both processes are induced by changes in plasma levels of gonadotropins. During their growth, oocytes acquire the ability to restart meiotic maturation in response to gonadotropins stimulation, mainly LH increased. The nuclear envelope breaks down, the first polar body is released and meiosis progress until metaphase II (MII). The meiosis stops in MII and ovulation occurs. Maturation promoting factor (MPF) and mitogen activated protein kinases (MAPK) play an important role in oocyte maturation.
Study design, size, duration: Prospective comparative study. Fifty couples who underwent ART were selected. From January 2014 to December 2015.
Participants/materials, setting, methods: From nineteen patients, 22 metaphase II and 18 unfertilized oocytes after ICSI were studied.

The first polar body was collected for chromosomal analysis by aCGH.

Oocytes were processed by immunocytochemistry-ICC to determine oocyte maturation: assessment of inactive MPF status and the metaphase plate alignment.

Other 31 couples with sub-optimal fertilization (<50%) were selected: unfertilized oocytes were studied by ICC. Two groups were conformed according to the main feature observed: (A) cytoplasmic immaturity and (B) mature cytoplasm.
Main results and the role of chance: Regarding MII mature oocytes, 87% had a normal metaphase plate and 84% were chromosomally normal. Contrary, immature oocytes presented abnormal metaphase plate (86%) and just 33% were euploid.

In failed-fertilized oocytes: 100% of mature oocytes had a normal metaphase plate and 71% were euploid. When oocytes were cytoplasmic immature, 37% of them were normal (metaphase plate) and 50% were chromosomally normal.

The global rate of aneuploidies and metaphase plate disarrangements in immature oocytes (MII + failed-fertilized) were significantly higher than mature oocytes ($p < 0.05$).

In patients with sub-optimal fertilization, the percentage of top quality embryos and pregnancy rate was significantly higher in group B ($p < 0.05$).
Limitations, reasons for caution: More oocytes should be studied.
Wider implications of the findings: Our results suggest that cytoplasmic oocyte maturation has a higher relevance compared to nuclear maturity.

An inadequate cytoplasmic maturation is associated with altered levels of metaphase plate alignment and aneuploidies affecting pregnancy rates.
Trial registration number: None.

O-276 AMH levels, independent of age, are a significant marker for risk of miscarriage following post-IVF embryo transfer

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Study question: Maternal age is associated with both higher miscarriage rates and lower levels of AMH, however the individual contribution of AMH levels to miscarriage is unknown.
Summary answer: AMH levels are correlated with an increased miscarriage risk after IVF, independent of age.
What is known already: Advanced maternal age is a well established risk factor for pregnancy loss in general and after IVF treatment. Maternal age is also associated with lower markers of ovarian reserve such as AMH. It is less clear, however, how younger patients with abnormal markers of ovarian reserve, should be counseled with respect to the likelihood that a pregnancy will result in a loss.
Study design, size, duration: We performed a retrospective cohort study on patients who achieved pregnancy with IVF at 12 fertility treatment centers in the United States from 2009 to 2015. We excluded patients with ectopic pregnancies, cycles using donor oocytes or gestational carriers, and included patients 22–49 years old in which AMH testing had been performed. We included both fresh and frozen embryo transfer IVF and excluded cycles where PGS was performed. Our final analysis was performed on 13,463 patients.
Participants/materials, setting, methods: We analyzed 16,039 IVF cycles (corresponding to 13,463 patients), of which 10,748 cycles resulted in a live birth, 2,733 in a biochemical pregnancy loss (BP), and 2,558 in a clinical miscarriage (CM), defined as detection of a gestational sac by ultrasound. Time-dependent multivariate time-to-event models were used to evaluate the hazard (risk) of miscarriage for BP and CM when controlling for multiple clinical parameters. Predictors were refined using least absolute shrinkage and selection operator (LASSO).
Main results and the role of chance: Consistent with previous studies, we found that maternal age was a significant risk factor for both BP (4.3% increase/year, $P < 0.001$) and CM (6.9% increase/year, $P < 0.001$). We next explored the relationship between AMH levels and miscarriage risk. We found that risk of BP was significantly higher in patients with low AMH, independent of age. Patients with AMH less than 0.2 ng/mL were at a 29.1% higher risk of BP ($P = 0.01$) and patients with an AMH between 0.2 and 0.95 ng/mL had a 10.4% increased risk ($P = 0.051$). Additionally, we found that AMH was a significant predictor of risk of CM in patients with both very low and high AMH, independent of age. Patients with AMH less than 0.2 ng/mL had a 23.8% increased risk ($P = 0.034$), and, conversely, patients with AMH that was greater than 10 ng/mL had a 25.6% increased risk of CM ($P = 0.02$). Interestingly, for patients with AMH between 0.2 and 0.95 ng/mL, increased risk is dependent on maternal age, where each year increase in age results in a 3.2% increase in risk for CM ($P = 0.015$).
Limitations, reasons for caution: Our study was performed on retrospective data from the U.S. Future studies are needed to investigate whether findings could expand to European practice patterns. We also excluded cycles in which PGS was performed. Inclusion of PGS in future work, may better resolve the etiology of pregnancy loss in these groups.
Wider implications of the findings: Our study suggests that AMH is a powerful biomarker for determining risk of miscarriage during IVF treatment. Women who are at risk for miscarriages either due to abnormally low or high AMH level, independent of age, may benefit from increased counseling.
Trial registration number: None.

SELECTED ORAL COMMUNICATIONS

SESSION 71: THE COMPROMISED OVARY

Wednesday 06 July 2016 **Hall 5 A** **14:00–15:15**

O-277 The effect of GV1001 and bevacizumab combination therapy on follicle loss of ovary

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Study question: Can the combination therapy of GV1001 and bevacizumab effects on function of ovary and suppression of lung cancer?
Summary answer: GV1001 and bevacizumab combination therapy prevents follicle loss of ovary.
What is known already: Premature ovarian failure and infertility are major side effects of chemotherapy treatments in young cancer patients. A more thorough understanding of the mechanism behind chemotherapy-induced follicle loss is necessary to develop new methods to preserve fertility in these patients. Bevacizumab (Avastin®), a monoclonal antibody directed against VEGF, has shown promise in treating a variety of cancers. GV1001 is a 16-amino acid peptide vaccine derived from the human telomerase transcriptase (hTERT) sequence. It was developed as an anti-cancer agent. There are no report about the effect of these two combination therapy on lung cancer and the effect of normal ovarian function.
Study design, size, duration: Experimental animal study, 4 weeks study duration.
Participants/materials, setting, methods: Seven-week-old BALB/c athymic (Nu/Nu) mice (3 mice per group, 12 female, Orient Bio Co. Gyunggido, Korea) were inoculated with non small cell lung cancer cell lines (H441) subcutaneously, and then randomly divided into four groups. Mice were injected with PBS, GV1001 (50 μg/kg in 100 ml 0.9% NaCl solution), bevacizumab (2 mg/kg), or GV1001 plus bevacizumab twice a week for 4 weeks. Tumor sizes and growing follicle ratio of ovary were measured.
Main results and the role of chance: We show that the bevacizumab activates the growth of the quiescent primordial follicle population in mice, resulting in loss of ovarian reserve. Coadministration of a GV1001 reduced follicle activation and increased follicle reserve. These findings suggest that the mechanism in bavacizumab-induced loss of ovarian reserve is accelerated primordial follicle activation, which results in a "burnout" effect and follicle depletion. By preventing this activation, GV1001 shows potential as an ovarian-protective agent, which may be able to preserve fertility in female cancer patients.
Limitations, reasons for caution: Small number of subjects and only follicle counts of ovary are limitation of this study. We are going to test serum level of AMH, FSH, estradiol and VEGF. Further we will observe immunohistochemistry on tumor cells and ovary to make sure the effects of preserving effect and tumor suppression effect.
Wider implications of the findings: This is the first study by combination of GV1001 and bevacizumab in lung cancer mouse model.
Trial registration number: This is not a clinical study.

O-278 Novel microRNAs (miRNA) as biomarkers for toxic insult in the ovary

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Study question: Are there miRNAs exclusive to toxic insult in the ovary and how are they associated with dysregulation of cellular death and increased loss of follicles?
Summary answer: There are miRNAs induced by toxic insult of the ovary that are associated with regulation of autophagy.
What is known already: It is estimated that 50–80 million people worldwide are infertile. Environmental toxicants and life-style factors are thought to adversely affect female fertility in part via impaired ovarian function. We have used the cigarette smoke (CS) exposure to model ovarian insult and to explore mechanisms of ovarian follicle loss. We have shown that CS-exposure induces autophagy and decreases the number of primordial and growing follicles. miRNAs are small noncoding RNAs that negatively regulate gene expression post-transcriptionally and involved in a number of cellular processes. The regulatory role of miRNAs in the ovary and their potential in fertility preservation is not well-known.
Study design, size, duration: We used our well-established CS-exposure model to investigate ovarian toxicity in C57BL/6 mice. Eight week old mice were exposed to CS (treatment; $n = 4$) or room air (control; $n = 4$) twice daily for 5 days/week for a period of 8 weeks. miRNAs were isolated from ovarian homogenates and profiled using a miRNA Array (Qiagen and SAbiosciences, Canada) to quantify the expression of the 940 most abundantly expressed miRNAs found in the mouse miRNA genome.
Participants/materials, setting, methods: Differentially expressed miRNAs with a statistically significant fold-change (p value ≤ 0.05) were grouped according to increasing/decreasing expression. Nine miRNAs were selected for qPCR validation; miR-let-7, miR-221, miR-1a-1-5p, miR-155-3p, miR-125b-1-3p, miR-15b-5p, miR-451a, miR-1b-3p and miR-101a-3p. To further understand biological significance, a list of mRNA targets for each miRNA was generated utilising an online miRNA-target resource program. Additionally, functional analysis was investigated to identify pathways central to toxic insult-induced dysregulation of ovarian function and autophagy.
Main results and the role of chance: A total of 162 miRNAs were differentially expressed with fold changes greater than 2. Ten miRNAs had increasing fold-change and 152 miRNAs had decreasing fold-change. Both the array and qPCR validation revealed increased expression of miR-let-7 and miR-1a-1-5p and decreased expression of miR-125b-1-3p. miR-let-7 had the greatest overall change in expression, and studies have shown that miR-let-7 is implicated in ovarian cancers through the regulation of autophagy. Comparably, our previous investigations have shown that CS exposure causes increased ovarian follicle loss which is mediated by autophagy, as shown by increased expression of autophagy markers (Beclin1 and LC3) and decreased expression of autophagy inhibitors (mTOR). The association between let-7e and autophagic signalling further elucidates the complex molecular mechanisms associated with ovarian toxicity and the additional functional and enrichment analysis will establish the role of the selected miRNAs with respect to function and disease.
Limitations, reasons for caution: Although the results from the miRNA Array are indicative of biological relevance, further study of the implicated pathways is required. It is not currently known if similar miRNA are expressed and can be detected in human follicular fluid of women who smoke vs. non-smokers.
Wider implications of the findings: We previously indicated that dysregulation of reparative autophagy is a sensitive marker of ovarian toxic insult and an important therapeutic target for fertility preservation. We predict that results of the current study will assist in identifying markers of ovarian health to guide oocyte selection for fertilization to maximize fertility potential.
Trial registration number: N/A.

O-279 Impact of environmental endocrine disruptor's exposure in controlled ovarian stimulation outcome in egg donors

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Study question: Does the endocrine disrupting compounds (EDCs) exposure affect controlled ovarian stimulation outcome in egg donors?
Summary answer: We found significant correlations between lifestyle EDCs exposure, levels measured in urine and follicular fluid and controlled ovarian stimulation outcome in egg donors.
What is known already: Endocrine disruptors are chemicals that can interfere with the endocrine system. Found in many household and industrial products EDCs interfere with the synthesis, secretion, transport, binding, action, or elimination of natural hormones. The central role of the estrogens in the reproductive system has suggested that environmental EDCs exposure that imitates the biological estradiol effect could be a cause for reproductive disorders. Several EDCs have been associated with infertility in women. The most studied EDCs are bisphenol A, parabens and phthalates. We also included caffeine, cotinine and isoflavones such as daidzein and genistein in our study.
Study design, size, duration: Prospective, observational study including female egg donors with proven fertility attending our IVF institution from December 2013 until July 2015. Two urine samples (5 days spaced) and follicular fluid during oocyte retrieval were collected and all the EDCs were quantified. Stimulation parameters were registered and statistical analysis were performed. The following parameters were considered: number of antral follicles, E2 (hCG day), total FSH administered, number of oocytes and MII retrieved.
Participants/materials, setting, methods: 31 female donors were recruited for the study. Lifestyle exposure to EDCs were evaluated using a 40 item questionnaire filled by all donors recruited. Two urine samples (5 days spaced) and follicular fluid (FF) during oocyte retrieval were collected from all the donors. Bisphenol A (BPA), methyl-paraben (mPb), propyl-paraben (pPB), Mono (2-etil-hexil) phthalate (MEHP), caffeine, cotinine, genistein and daidzein (soy-derived phytoestrogens) levels were measured in both urine and follicular fluid using UPLC-MS.
Main results and the role of chance: This study correlates for the first time a panel of environmental EDCs lifestyle exposure and the EDCs measured in urine and follicular fluid with the clinical outcome of controlled ovarian stimulation outcome in egg donors. We detected all the EDCs compounds studied in urine and follicular fluid. We found a positive significant correlation between caffeine levels in both urine and follicular fluid and the total number of oocytes ($p = 0.002$ urine and $p = 0.004$ for follicular fluid) and MII retrieved ($p = 0.005$ urine and $p = 0.008$ for follicular fluid). We also observed a significant increase in the number of antral follicles with the soy-related food products ingestion ($p = 0.0011$) in our female donors. A significant correlation between the habit of reheat food in plastic containers and the number of antral follicles in these women were also found ($p = 0.048$). Finally, we also observed a significant correlation between total FSH levels and the habit of store food in plastic containers ($p = 0.040$).
Limitations, reasons for caution: Our main limitation is the low number of donors used in the study and the lack of external validation of our results. Furthermore, our results were conditioned by the limit of detection of the technique used for each analyte.
Wider implications of the findings: We are highly exposed to several EDCs found in our daily routine work, environment and food that could be found in the urine and follicular fluid altering egg quality and stimulation parameters. More attention to this EDCs exposure is needed to avoid detrimental effects in IVF donors and patients.
Trial registration number: NA.

O-280 Random-start ovarian stimulation in patients without cancer: comparison between conventional-start and random-start in different ovarian reserve

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Study question: To compare total amount of gonadotropins, stimulation length, number of retrieved oocytes and mature oocytes, fertilization rate, and clinical pregnancy rates between random-start and conventional-start.
Summary answer: Random-start is similar to conventional-start in terms of the number of retrieved oocytes and mature oocytes. Pregnancy rate was extremely high in patients with random-start.
What is known already: Random-start ovarian stimulation has usually been applied in patients with cancer for emergency fertility preservation. In such patients, utilizing their oocytes for fertilization and implantation is some years later after survival of cancer. Therefore, there are few studies to show fertilization, pregnancy, implantation and live birth rates just after oocyte retrieval. There are some patients who required fresh TESE-ICSI (oocyte retrieval and TESE are performed in the same day), whose viable sperms are predicted as very few and no longer tolerable after freeze and thaw if we can recover the sperms. Random-start ovarian stimulation might be useful in these patients.
Study design, size, duration: Our institute has dealt with more than 300 TESEs per year and more than 200 TESE-ICSI for past 2 years. We performed a retrospective cohort study between March 2014 and December 2015. We compared total amount of gonadotropins, stimulation length, number of retrieved oocytes and mature oocytes, fertilization rate, and clinical pregnancy rates between random-start (60 patients, 64 cycles) and conventional-start (149 patients, 162 cycles) as age-matched control. We included only patients without cancer.
Participants/materials, setting, methods: In random-start ovarian stimulation, the starting day was anytime except for during menstruation. In both groups, controlled ovarian stimulation was performed in the same manner. Less than 42 old and AMH >1.1 ng/mL was included in the study. We divided into two groups with ovarian reserve (AMH 1.1~4.0 and >4.0 ng/mL). Student's *t* test (non homogeneity, two-sided) was used for comparison between two groups. Chi-squared test was used for 2×2 contingency table.
Main results and the role of chance: The study population was limited to ICSI and freeze-all cycles only. In patients with AMH >4.0, age (32.4 vs. 33.6 years), AMH (8.0 vs. 6.9 ng/mL), total amount of gonadotropins (2294 vs. 2051 IU), stimulation length (10.1 vs. 8.4 days), number of retrieved oocytes (17.3 vs. 14.4) and mature oocytes (13.1 vs. 10.6), and fertilization rate (65.4 vs. 63.5%) were not different between random-start and conventional-start, respectively. Clinical pregnancy rate (80.0 vs. 35.7%), however, was significantly better in random-start than in conventional-start ($P = 0.001$). In patients with AMH 1.1–4.0, age (33.8 vs. 35.5 years), AMH (2.7 vs. 2.3 ng/mL), number of retrieved oocytes (9.2 vs. 8.7) and mature oocytes (6.4 vs. 6.3), fertilization rate (67.8 vs. 64.1%), and clinical pregnancy rate (35.0 vs. 24.3%) were not different between random-start and conventional-start, respectively. While total amount of gonadotropins (2912 vs. 2539 IU) and stimulation length (10.3 vs. 8.8 days) were significantly more in random-start than in conventional-start ($P < 0.001$). Although random-start has been reported to need 2 days more stimulation than conventional-start, this is applied to moderate ovarian reserve (AMH 1.1–4.0). With this additional stimulation period, oocytes were recovered as same as conventional-start.
Limitations, reasons for caution: Since this is a retrospective study, a prospective study is necessary for final conclusion. Because most patients in random-start are fresh TESE-ICSI patients and most patients in conventional-start are not, the former may not include female factor and the latter may include unexplained infertility, which relates to pregnancy rate.
Wider implications of the findings: This is the first study to show the relevance of random-start stimulation in patients without cancer. Random-start stimulation can be performed as same as conventional-start in patients with good ovarian reserve (AMH >4.0) such as patients who required fresh TESE-ICSI, and also in patients with moderate ovarian reserve (AMH 1.1–4.0).
Trial registration number: N/A.

O-281 Estimation of ovarian response with changing gonadotropin doses: a study of 14,805 women with repeated stimulation cycles

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Study question: What are the changes in ovarian response in women with repeated ovarian stimulation cycles?
Summary answer: An increase in gonadotropin dose can improve ovarian response when fewer (<11) oocytes are retrieved in the initial cycle.
What is known already: Ovarian response can be quantified by dividing the total gonadotropin stimulation dose by number of oocytes retrieved (dose/oocyte); a lower dose per oocyte indicates a better response than a higher dose per oocyte. Research from our group has shown negative association between dose/oocyte and success rates. There is ongoing debate on the optimal gonadotropin dose for ovarian stimulation. Oocyte numbers do not always increase with higher gonadotropin doses, and it is not known how to estimate the change in ovarian response for individual patients. Therefore, in current practice, the change in dose from one cycle to the next varies considerably.
Study design, size, duration: Retrospective cohort study of 29,610 ovarian stimulation cycles for non-donor IVF/ICSI performed in 2008–2012 in the U.S. (National Assisted Reproductive Technology Surveillance System, $n = 26{,}996$) and Finland (LUMI database, $n = 2{,}614$). Included were first and second cycles of women treated twice with the same protocol [long gonadotropin-releasing hormone (GnRH) agonist or GnRH-antagonist protocol], with change in total gonadotropin dose in the second cycle between 4,000 and 5,000 IU and with ≥4 oocytes retrieved in both cycles.
Participants/materials, setting, methods: In all, 9,175 women were treated with the long and 5,630 with the antagonist protocol. Data were analyzed separately by protocol. We examined the relationship between number of oocytes retrieved, total gonadotropin dose and total gonadotropin dose/oocyte, and the change in total gonadotropin dose from the first to the second stimulation cycle. Results were stratified by number of oocytes retrieved in the first cycle (4–10 vs. ≥11 oocytes).
Main results and the role of chance: Total gonadotropin dose per oocyte showed a non-linear increase with higher stimulation dose. Increases were more pronounced and started at lower doses in cases with lower oocyte numbers as dose/oocyte was inversely related to the number of oocytes retrieved. In women stimulated with the long protocol, mean stimulation dose increased from 2,399.6 ± 1,995.5 to 2,606.8 ± 1,254.3 IU ($P < 0.0001$), the number of oocytes retrieved increased from 14.4 ± 6.8 to 14.8 ± 6.9 ($P = 0.02$), and dose/oocyte increased from 212.7 ± 180.9 to 230.9 ± 196.1 IU ($P < 0.0001$). Starting stimulation dose was higher in U.S. than in Finnish women (2383.5 ± 1182.2 vs. 2048.7 ± 795.6 IU, $P < 0.0001$). Results in antagonist cycles were similar.

The change in dose/oocyte differed based on the initial number of oocytes retrieved. In women with 4–10 oocytes retrieved, an increase of dose up to 530 (antagonist) and 600 IU (long protocol) was associated with improved ovarian response (lower dose/oocyte). With further increase of stimulation dose, response gradually worsened. In contrast, in patients with ≥11 oocytes retrieved, any increase in dose was associated with poorer ovarian response (higher dose/oocyte).
Limitations, reasons for caution: The second stimulation took place on average 0.6 (antagonist protocol) and 1.3 (long protocol) years after the first one. Despite the big difference in the number of cycles and different stimulation doses, the relationship between stimulation dose and dose/oocyte among U.S. and Finnish patients was similar.
Wider implications of the findings: Our findings may help in tailoring ovarian stimulation for patients with unsuccessful first treatment, especially when trying to estimate individual maximal gonadotropin doses. Future studies could account for differences in ovarian response by comparing treatment outcomes according to dose/oocyte values.
Trial registration number: None.

SELECTED ORAL COMMUNICATIONS

SESSION 72: THE SURROGATE AND PROSPECTIVE PARENTS EXPERIENCE OF SURROGACY

Wednesday 06 July 2016 **Hall 3 AB** **14:00–15:15**

O-282 Efficacy of a short educational video on fertility knowledge and intentions over 12 months: a randomised controlled trial in young adults.

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Study question: What are the effects of a short educational video in increasing fertility knowledge and intentions on young adults over the course of 12 months?
Summary answer: A brief inexpensive educational video increases knowledge and intended age for bearing the last child (but not the first), with most changes sustained over time.
What is known already: Young adults have low fertility knowledge, particularly regarding the effect of age on fecundity and ART success rates. Hence, the increasing childbearing postponement we have been witnessing in Europe for more than 50 years might not be entirely conscious, and raising awareness is imperative. Recent evidence shows that information increases fertility knowledge, but the long-term effectiveness has been seldom analysed, with no evidence regarding the use of RCTs or 1-year follow-ups. Additionally, the efficacy of a video-based intervention has never been evaluated, and these are typically more accessible in terms of language and communication and more cost-effective than written information.
Study design, size, duration: A prospective, two-arm, parallel group, single blind randomized controlled trial was performed between October 2014 and November 2015. A sample size of 73 participants per group (85% power to observe a significant difference $\alpha = 0.05$) was estimated. Participants ($n = 269$) were randomly allocated into intervention group (IG, fertility awareness video) and control group (CG, no intervention), and were assessed before (T0), immediately after (T1), 1-week (T2), 1-month (T3), and 1-year (T4) after the intervention.
Participants/materials, setting, methods: Participants were undergraduates aged 20 years (SD = 2.69) who completed self-report questionnaires including sociodemographic, fertility knowledge and childbearing intentions questions at all time-points. Participants in the IG were exposed to a 2-min video giving information on infertility definition and risk factors, and pregnancy chances according to women's age. Participants in the CC received no stimulus. Mixed models ANOVA with Bonferroni *post-hoc* comparison tested interaction, time and group effects on fertility knowledge and intention variables.
Main results and the role of chance: Subjects were excluded if they declared knowledge of a fertility problem ($n = 2$), being parents ($n = 10$), or were outliers ($n = 2$), leaving 255 subjects, 137 IG and 118 CC. Using a criterion of ≥3 moments within each outcome, missing values were handled using multiple imputation. There were no pretest differences among groups. A significant interaction was found between assessment time and group on knowledge variables (infertility definition; infertility risk factors; spontaneous pregnancy chance; and treatment success, Ps < 0.001) and on desired age for bearing the last child ($P < 0.05$). No significant interaction or main effects were found for the intended age of first child. The IG significantly increased awareness from pre to post test in all knowledge variables, and while there were significant decreases over time, values were still significantly higher compared to CC knowledge at all follow-ups. However, knowledge on female infertility risk factors was not sustained after 1 week. Regarding intentions, while there were no changes in both groups on the intended age of first child, IG subjects significantly reduced their intended age of last child, and this intention was maintained 1 year after visualizing the video. There were no significant differences over time within the CC.
Limitations, reasons for caution: Caution should be exerted when generalizing to older populations or subjects not attending college, as knowledge and

intentions might be different and alternative interventions might be more effective in these groups.
Wider implications of the findings: This study has shown the potential of a 2-min video in reaching a massive population of technology and media users, thus facilitating higher education students' reproductive health empowerment and behaviour change. Further studies should test the influence of knowledge in reproductive behaviours and decisions and analyse potential mediators.
Trial registration number: Clinicaltrials.gov NCT02607761.

O-283 The UK longitudinal study of donor insemination, egg donation and surrogacy families: a follow-up at adolescence

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[1]*University of Cambridge, Centre for Family Research, Cambridge, UK*

Study question: Do families formed through reproductive donation differ from natural conception families in psychological well-being and the quality of parent–child relationships as the children enter adolescence?
Summary answer: All family types were functioning well. Differences reflected particularly positive parent–adolescent relationships in surrogacy families. Egg donation families showed the least positive relationships.
What is known already: It has been suggested that reproductive donation may lead to detrimental outcomes for parent–child relationships and child adjustment due to the absence of a genetic and/or gestational link between parents and children. Although previous phases of this study when the children were aged 1, 2, 3, 7 and 10 years found positive outcomes in family functioning, adolescence may present particular challenges for these families as this is the developmental stage at which issues relating to identity and autonomy become salient and difficulties in parent–child relationships are most likely to arise.
Study design, size, duration: This is the sixth phase of a longitudinal study of surrogacy families, egg donation families, donor insemination families and a comparison group of natural conception families that commenced in 2000.
Participants/materials, setting, methods: This study uses a multi-method, multi-informant approach to data collection. Data were collected from mothers and their adolescent children in their homes and comprised a standardised interview of parenting quality, questionnaires of parent-adolescent relationships and a video-recorded observational task. At the present phase, data were obtained from 32 donor insemination, 27 egg donation, 28 surrogacy, and 54 natural conception families, representing 91, 84, 90 and 100% respectively of participants and Phase 5.
Main results and the role of chance: Interview data were transformed into factor scores of Positive Parenting (warmth, quality of interaction, and sensitive responding) and Negative Parenting (frequency and level of battle, and resolution). There was no difference between family types in Positive Parenting. However, a difference was found for Negative Parenting, F (3, 137) = 12.91, $p < 0.01$, reflecting less negative parenting in the surrogacy families than in the gamete donation families. There was also a difference for the Index of Family Relationships as rated by both mothers and adolescents, F (6, 184) = 5.11, $p < 0.001$, reflecting fewer family relationship difficulties in surrogacy families compared to gamete donation families, and greater family relationship difficulties in egg donation families compared to donor insemination families. Similarly, there was a difference in parental acceptance, F (6, 184) = 3.06, $p < 0.05$, indicating greater maternal acceptance as rated by mothers in surrogacy families compared to gamete donation families, and lower maternal acceptance in egg donation families compared to donor insemination families. There were no differences between family types for the observational measure of mother-adolescent interaction or adolescent adjustment. Longitudinal path analyses indicated stability in parent-child relationships from age 7 to 14 years.
Limitations, reasons for caution: The relatively small sample sizes may mean that differences between family types were not identified due to insufficient statistical power. Also, attrition may have resulted in sample bias such that families experiencing difficulties may have been more likely to drop out.
Wider implications of the findings: Contrary to concerns about potentially negative long-term psychological consequences, the findings of this longitudinal study indicate that families formed by reproductive donation, particularly surrogacy families, continue to function well when the children enter adolescence despite the additional challenges that these families face.
Trial registration number: None.

O-284 Gay men's journeys to parenthood via surrogacy: an exploratory study of UK residents

W. Norton[1], N. Hudson[1], J. Fish[1], L. Culley[1]
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Study question: What are the motivations and experiences of UK resident gay men who have become fathers, or are considering fatherhood, via surrogacy?
Summary answer: Surrogacy is a complex, multi-faceted route to fatherhood. Men's decision-making is fluid and contextual. Men sought genetic and gestational arrangements in UK and cross-border settings.
What is known already: The number of people having children in the context of a lesbian or gay identity is increasing. However, the evidence available is disproportionately devoted to the experience of lesbian mothers. The limited research on gay fathers has primarily focussed on quality of parenting or transition to parenthood, and largely related to men who have become parents through adoption, co-parenting or through previous heterosexual relationships. There is a dearth of literature on gay men's desire and decision to parent, and few studies focusing on surrogacy as a pathway to parenthood; existing work is focused on the USA and Australia.
Study design, size, duration: This exploratory, qualitative study examined the perspectives of UK resident men. Data regarding the participants' motivations and experiences of surrogacy were collected from a purposive sample of 21 men by means of in-depth, semi-structured interviews conducted between May 2013 and November 2014.
Participants/materials, setting, methods: Fourteen in-depth, face-to-face interviews were conducted with 21 men. Eleven of these interviews were with men who had already become fathers via surrogacy, (7 couple interviews, 4 individual interviews), and the three remaining interviews were with men considering surrogacy (3 individual interviews). Interviews were recorded and transcribed verbatim. Data were analysed in NVivo10 using a systematic, thematic method, informed by an interpretivist approach.
Main results and the role of chance: The presentation reports on demographic characteristics of the participants, their motivation for choosing this specific route to become fathers, the type of surrogacy undertaken, their rationale for accessing surrogacy within the UK or overseas and their experiences of surrogacy. Men's journeys to parenthood via surrogacy were found to be complex and challenging. The participants engaged in multiple surrogacy arrangements in both UK and cross-border settings in their journey to become fathers. These arrangements are very much "planned" pregnancies; however men reported requiring a high level of resilience to achieve their desire of a family. The findings indicate that gay men's decision-making is fluid and contextual, and their needs and priorities change in relation to the length of time it takes to achieve a pregnancy.
Limitations, reasons for caution: The study's small, self-selecting sample limits the generalisation of the findings. However, to the authors' knowledge, no other research has yet to examine the perspectives of gay men choosing surrogacy to become parents within the UK context. This research is therefore an important addition to the limited empirical knowledge base.
Wider implications of the findings: A rapidly increasing number of gay men are exploring the possibility of surrogacy as a means of creating a family. These findings offer insights that may inform the development of policies and practice that are better able to support the needs of gay men throughout their surrogacy journey.
Trial registration number: N/A.

O-285 When the womb is overseas: the meaning-making of the gestational surrogacy in gay couples

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Study question: How do gay couples deal with gestational surrogacy overseas?
Summary answer: Feelings of impotence due to the distance, the importance of a close relationship with the gestational carrier, and gratitude for pregnancy emerged.
What is known already: In the last two decades an increasing number of gay men has entered into surrogacy arrangements to become parents. Previous studies have addressed various aspects of this process, including changes associated with the transition to parenthood, the effect of upcoming fatherhood on how coping with the seemingly contradictory identities as gay and as parents, the motivations for seeking extraterritorial surrogacy, and the ethical issues regarding the potential exploitation of the women involved. However, there remains a dearth of studies that deals with the psychological implications of the physical distance between the gay fathers and the gestational carrier during the pregnancy.
Study design, size, duration: Cross-sectional study. Data were collected between February and August 2015 on 20 gay-partnered fathers who pursued gestational surrogacy overseas in America or Canada because in Italy surrogacy is prohibited by law. Gay fathers were eligible for the study if they were Italian, had at least one child born in their current relationship, and did not receive a friend or relative's oocyte donation.
Participants/materials, setting, methods: Gay fathers were recruited via the Italian Rainbow Families. Joint, semi-structured interviews took place at fathers' home and lasted 60–90 min. After the transcription, a draft was sent to each participant for amendment and approval, often with additional questions. An Interpretative Phenomenological Analysis was performed by two independent coders to identify recurrent themes. Critical feedback was discussed during all phases of analysis in team meeting.
Main results and the role of chance: This study showed three recurrent themes in how Italian gay fathers deal with their gestational surrogacy arrangement overseas. All themes are related to consequences of the distance between fathers and the physical pregnancy. The loss of a sense of control with the subsequent feelings of frustration and anxiety is the main implication of the inability to experience the physical presence of the fetus. As consequence, fathers highlighted the importance of establishing a close relationship with the gestational carrier also to be always posted about ultrasounds and test results. Most couples reported that their emotional involvement is mirrored by the gestational carrier, who want to be called "special auntie" for including in the relationship also their husbands and children. Finally, fathers represented their gestational carrier with images of hospitality and force of nature, expressing a deep sense of gratitude for making them parents.
Limitations, reasons for caution: Conjoint interviews process could have affected fathers' personal points of view, hindering their ability to express themselves if they perceived their opinions as inconsistent with those of their partner. Moreover, retrospectively constructed stories do not allow for an extrapolation of the conclusions made.
Wider implications of the findings: Our findings help in developing strategies to counsel Italian gay parents who experience distance with the gestational carrier and the fetus. The value of defining international guidelines for cross-border reproductive services is also supported at the aim of handling the uncertainty, unease and potential complications arising from the surrogacy process.
Trial registration number: N/A.

O-286 A longitudinal study of the experiences and psychological well-being of Indian surrogates

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Study question: What is the psychological well-being of Indian surrogates during and after the surrogacy pregnancy?
Summary answer: Surrogates were similar to a matched group of expectant mothers on anxiety and stress. However, they scored higher on depression during and after pregnancy.
What is known already: The recent ban on trans-national commercial surrogacy in India has led to urgent policy discussions regarding surrogacy. Whilst previous studies have reported the motivations and experiences of Indian surrogates no studies have systematically examined the psychological well-being of Indian surrogates, especially from a longitudinal perspective. Previous research has shown that Indian surrogates are motivated by financial payment and may face criticism from their family and community due to negative social stigma attached to surrogacy. Indian surrogates often recruited by agencies and mainly live together in a "surrogacy house."
Study design, size, duration: A longitudinal study was conducted comparing surrogates to a matched group of expectant mothers over two time points: (a) during pregnancy (Phase1: 50 surrogates, 70 expectant mothers) and (b) 4–6 months after delivery (Phase 2: 45 surrogates, 49 expectant mothers). The Surrogates were recruited from a fertility clinic in Mumbai and the matched comparison group was recruited from four public hospitals in Mumbai and Delhi. Data collection was completed over 2 years.
Participants/materials, setting, methods: Surrogates and expectant mothers were aged between 23 and 36 years. All participants were from a low socio-economic background and had left school before 12–13 years of age. In-depth face-to-face semi-structured interviews and a psychological questionnaire assessing anxiety, stress and depression were administered in Hindi to both groups. Interviews took place in a private setting. Audio recordings of surrogate interviews were later translated and transcribed into English.
Main results and the role of chance: Stress and anxiety levels did not significantly differ between the two groups for both phases of the study. For depression, surrogates were found to be significantly more depressed than expectant mothers at phase 1 ($p = 0.012$) and phase 2 ($p = 0.017$). Within the surrogacy group, stress and depression did not change during and after pregnancy. However, a non-significant trend was found showing that anxiety decreased after delivery ($p = 0.086$).

No participants reported being coerced into surrogacy, however nearly all kept it a secret from their wider family and community and hence did not face criticism. Surrogates lived at the surrogate house for different durations. During pregnancy, 66% ($N = 33/50$) reported their experiences of the surrogate house as positive, 24% ($N = 12/50$) as negative and 10% ($N = 5/50$) as neutral. After delivery, most surrogates (66%, $N = 30/45$) reported their experiences of surrogacy to be positive, with the remainder viewing it as neutral (28%) or negative (4%). In addition, most (66%, $N = 30/45$) reported that they had felt "socially supported and loved" during the surrogacy arrangement by friends in the surrogate hostel, clinic staff or family. Most surrogates did not meet the intending parents (49%, $N = 22/45$) or the resultant child (75%, $N = 34/45$).
Limitations, reasons for caution: Since the surrogates were recruited from only one clinic, the findings may not be representative of all Indian surrogates. Some were lost to follow-up which may have produced sampling bias.
Wider implications of the findings: This is the first study to examine the psychological well-being of surrogates in India. This research is of relevance to current policy discussions in India regarding legislation on surrogacy. Moreover, the findings are of relevance to clinicians, counselors and other professionals involved in surrogacy.
Trial registration number: N/A.

SELECTED ORAL COMMUNICATIONS

SESSION 73: GONADOTOXICITY AND FERTILITY PRESERVATION

Wednesday 06 July 2016 **Hall 3 DE** **14:00–15:15**

O-287 ABVD chemotherapy for lymphoma affects number and morphology of primordial follicles in the adolescent and adult ovary

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Study question: Does the chemotherapeutic regimen of adriamycin, bleomycin, vinblastine and dacarbazine (ABVD), used to treat lymphoma, effect the number and development of ovarian follicles?

Summary answer: This study reports an increase in the number of non-growing follicles in the ovarian cortex of adolescents and adults previously treated with ABVD for lymphoma.

What is known already: Chemotherapy can compromise oocyte reserve resulting in reduced fertility potential. Treatment of lymphoma is determined by a number of factors including patient age, and type and stage of disease. Early stage Hodgkin's Lymphoma is commonly treated by ABVD, a non-alkylating regimen which has a low risk of premature ovarian insufficiency. Spontaneous ovulation, pregnancy and live births have been reported in patients treated with ABVD. Given the ovarian sparing qualities of ABVD it is important to investigate the histological appearance and distribution of follicles within ABVD-treated ovarian tissue obtained from young and adult patients.

Study design, size, duration: Histological analysis and immunohistochemical investigation of fixed human ovarian cortex from adolescent and adult women with lymphoma treated with ABVD, to ascertain developmental stage and concentration of follicles in comparison with untreated controls and samples from lymphoma patients treated with vincristine, etoposide, prednisone, doxorubicin (OEPA) and cyclophosphamide, vincristine, prednisone, dacarbazine (COPDAC). Human ovarian biopsies (n = 14, ages 12–30 years) were collected with informed consent and Ethical Committee approval.

Participants/materials, setting, methods: Cortical sections were analysed and follicle number and maturity evaluated. Only follicles with a visible germinal vesicle and nucleolus were evaluated to avoid over-counting. Tissue volume was calculated as the sum of the area of all analyzed sections per patient, multiplied by the section thickness. Mean follicle concentration was determined by dividing the total number of follicles per patient by the volume of tissue analyzed. Anti-DDX4 antibody was used to determine germ cell marker expression.

Main results and the role of chance: The majority of follicles in controls and ABVD tissue-treated were non-growing with <25% in controls and significantly fewer (<2%) in post-ABVD group at the primary stage or beyond ($p < 0.01$). Mean follicle concentration observed in pre-chemotherapy tissue closely matched predicted age-matched model-generated values whilst fewer follicles were present in patients treated with OEPA-COPDAC. Tissue from patients treated with ABVD had more than 3 times higher cortical follicle concentration than predicted ($p < 0.01$) with no evidence of increased growth initiation. The length of time between ABVD administration and biopsy collection from patients varied from 4 to 52 weeks. No correlation was detected between this interval and follicle numbers observed. Clusters of follicles and bi-ovular follicles, characteristics of fetal and infant ovaries, were frequently observed in ABVD-treated tissue. The germ cell marker DDX4 was localised in these clustered and multi-ovular structures, indicating that in the post-ABVD adolescent and adult ovary, the morphology of some follicles resembles that seen in infancy. These preliminary data suggest that unlike alkylating chemotherapeutic agents, ABVD treatment does not result in a reduction in the non-growing follicle population in the adult human ovary *in vivo* but may in fact cause an increase. The mechanisms for this are unclear at present.

Limitations, reasons for caution: The concentration of non-growing follicles was not depleted by ABVD in the adolescent and adult ovary, whereas other treatments showed the expected loss of follicle number. The appearance and distribution of non-growing follicles post-ABVD treatment resembled that of the infant human ovary. Mechanisms underpinning these observations are, as yet, undetermined.

Wider implications of the findings: This study corroborates reported ovarian sparing qualities of ABVD, confirming no depletion of non-growing follicles following ABVD. This is the first report of a morphological similarity between post-ABVD treated adult tissue and infant human ovary. Future experiments will investigate the basis for the apparent increase in follicle number post-ABVD.

Trial registration number: N/A.

O-288 Pharmacological administration of ceramide-1-phosphate (C1P) prevents chemotherapy-induced premature ovarian failure (POF)

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Study question: We investigated whether the administration of the sphingolipid ceramide-1-phosphate (C1P) can preserve ovarian function from gonadotoxicity caused by cyclophosphamide (CTX) in a mouse model.

Summary answer: C1P protects against CTX-induced gonadotoxicity by preserving the ovarian reserve and contributing to blood vessel formation, providing a novel strategy to avoid chemotherapy-induced POF.

What is known already: Premature ovarian failure (POF) is often a consequence of gonadotoxic chemoradiotherapy. Depleted follicle reserve can present with transient or permanent amenorrhea, infertility and premature menopause. In particular, alkylating agents such as cyclophosphamide (CTX) induce severe follicle loss, the proposed mechanisms being apoptosis and/or damage to ovarian microvascularization. Bioactive sphingolipids, such as C1P, are important regulators of cell homeostasis. As well as sphingosine-1-phosphate (S1P), C1P has a proangiogenic and anti-apoptotic role, with the advantage of a greater stability. Since the efficacy of gonadotropin-releasing hormone analogs is still controversial, we propose C1P as a potential protective molecule for fertility preservation.

Study design, size, duration: Twenty-four 8-week-old female F1 mice (BALB/c × C57BL/6) were separated into four groups (n = 6/group). Mice received either a single intraperitoneal injection of saline (control group) or 75 mg/kg of CTX. Besides, CTX mice received intrabursal administration of vehicle (CTX group) or C1P in two different doses (CTX+C1P groups: 5 µl/ovary; 0.5 mM or 10 µl/ovary; 0.6 mM). Animals were euthanized on day 14, and their ovaries removed and fixed in Bouin's solution for further study.

Participants/materials, setting, methods: Bouin-fixed, paraffin-embedded slides of ovarian tissue were stained with hematoxylin and eosin (H&E) and the number of different stages of follicles was determined in 3 sections per ovary. Data are expressed as the percentage of each follicle type per ovary. Ovaries were also immunostained for anti-Müllerian hormone (AMH) and for vonWillebrand factor by immunohistochemistry (IHC). Quantification of relative vascular areas was performed with ImageProPlus software.

Main results and the role of chance: Firstly, we analyzed follicular structures in H&E-stained ovarian slides. In CTX-treated ovaries, percentages of primary and preantral follicles were lower (both $p < 0.01$) and the percentage of atretic follicles was higher ($p < 0.01$) when compared with control ovaries. Local administration of C1P in CTX-treated ovaries (both CTX + C1P groups) increased the percentage of primary and preantral follicles, and decreased the percentage atretic follicles compared to CTX alone ($p < 0.001$). No significant differences were observed in the% of corpora lutea between groups.

Next, we studied the protein expression of AMH, a well-known marker of ovarian function, by IHC. Consistent with follicular count results, AMH expression decreased in the CTX group compared to the control group, while both doses of C1P increased AMH expression in CTX-treated ovaries.

Furthermore, the IHC for Von Willebrand factor, an endothelial cell marker, showed decreased vascular area in CTX-treated ovaries compared to control ($p < 0.05$). Administration of C1P in both doses restored the vascular area to levels comparable to those of control ovaries ($p < 0.05$).

In all cases, one-way ANOVA, followed by Tukey comparisons, were performed to test for statistical significances. P values < 0.05 were considered significant.

Limitations, reasons for caution: Caution is required for translation of results to human patients. Possible doses and routes of C1P administration remain to be elucidated.
Wider implications of the findings: Our results suggest that C1P preserves ovarian reserve in CTX-induced POF by improving follicular dynamics and ovarian angiogenesis. Given the increasing number of cancer survivors, finding effective, low-cost fertility-extending options for women undergoing life-preserving treatments is critical. C1P could be a novel strategy for fertility preservation in cancer patients.
Trial registration number: NA.

O-289 Evidence that administration of an antioxidant with anticancer properties can prevent cyclophosphamide gonadotoxicity by modulating early ovarian response to oxidative stress

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Study question: This work investigates whether oral administration of a natural carotenoid affects ovarian early response to cyclophosphamide (CPM) and prevents gonadotoxicity in female mice.
Summary answer: Crocetin administration prior to CPM prevents gonadotoxic effect on ovarian reserve by modulating SIRT1 levels and mitochondrial damage.
What is known already: Although oxidative stress (OS) is considered one of the mechanisms involved in CPM ovarian toxicity, the efficacy of *in vivo* antioxidant interventions has been poorly investigated. Dietary intake of some carotenoids, including saffron-derived crocetin, is known to exert potent anti-tumor effects and to protect non-malignant tissues against CPM toxicity throughout antioxidant and anti-inflammatory effects. An early sensor of OS is SIRT1, a deacetylase activated in response to changes in the redox state due to ROS increase and mitochondrial failure. By activating numerous targets SIRT1 orchestrates early response to OS and promotes cell survival or apoptosis.
Study design, size, duration: Eighteen CD-1 female mice were divided in three groups and received a single intraperitoneal injection of 100 µl of PBS (CTRL), or an equal volume containing CPM (100 mg/kg) (CPM) or crocetin extracted from saffron (100 mg/kg) *per os* for 15 days prior to CPM administration (Crocetin+CPM).
Participants/materials, setting, methods: Ovaries were analysed at 24 h post-CPM for SIRT1, SOD2, PGC1alpha, pAKT and pFOXO3a expression by Western blotting (WB). At 7 days post CPM ovaries were monitored for relative abundance of ovarian follicles by hematoxylin and eosin staining.
Main results and the role of chance: Crocetin administration prior to CPM-treatment decreased follicles loss and inhibited PI3K/PTEN/AKT pathway involved in activation of follicle recruitment. Primordial and antral follicles were significantly increased in the crocetin+CPM group when compared with CPM-mice (One-way ANOVA and Student–Newman–Keuls Multiple comparison $p < 0.001$). SIRT1 expression in CPM-mice was found to increase revealing the occurrence of OS. Mitochondrial superoxide dismutase SOD2 and the mitochondrial biogenesis activator PGC1alpha decreased suggesting that CPM-induced OS was associated with mitochondrial damage. Crocetin administration under conditions preventing follicle damage allowed the maintenance of basal levels of SIRT1 suggesting that preservation of redox balance can help the ovary counteracting ovarian damage by CPM. We also found that SOD2 and PGC1alpha were increased in crocetin + CPM mice providing evidence for mitochondrial protection.
Limitations, reasons for caution: The main limitation of this study was the absence of direct quantification of SIRT1 enzymatic activity. Results can be translated to humans with caution.
Wider implications of the findings: Our results aim to increase the knowledge of mechanisms underlying ovarian damage by CPM and will be helpful to develop new therapeutic opportunities for preserving fertility in cancer patients based on administration of antioxidants with anticancer properties. SIRT1 may be a biomolecular marker for evaluating cytotoxicity of other chemotherapeutic agents.
Trial registration number: Not required.

O-290 Seminiferous tubule integrity, Sertoli cell maturation and Leydig cell functionality after long-term organotypic culture of human immature testicular tissue

F. De Michele[1]
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Study question: Is an organotypic culture system able to provide the appropriate testicular microenvironment for *in vitro* maturation of human immature testicular tissue (ITT)?
Summary answer: Our organotypic culture system provided a microenvironment capable of preserving seminiferous tubule (ST) integrity and Leydig cell (LC) functionality, and inducing Sertoli cell (SC) maturation.
What is known already: Cryopreservation of human ITT containing spermatogonial stem cells (SSCs) is a well-established strategy to preserve fertility in prepubertal boys facing cancer, with a view to obtaining sperm. While in mice spermatogenesis has been replicated in organotypic culture, yielding reproductively efficient spermatozoa, this process has not been achieved in humans as yet. Only a few steps have been completed with adult testicular tissue, with differentiation of preleptotene spermatocytes into early spermatids over the course of 16 days of culture, despite considerable loss of germ cells. To date, no culture system has been described for human ITT.
Study design, size, duration: The study was designed to attempt to *in vitro* mature frozen–thawed ITT. For this purpose, 1 mm^3 tissue fragments from three prepubertal patients aged 2 (P1), 11 (P2) and 12 (P3) years were placed in organotypic culture for 139 days. Culture media, supplemented with either human chorionic gonadotropin (hCG) or testosterone, were compared.
Participants/materials, setting, methods: ST integrity and tissue viability were assessed by histological score and lactate dehydrogenase (LDH) dosage in supernatant. MAGE-A4 and Ki67 immunohistochemistry (IHC) identified spermatogonia, proliferating cells and proliferating spermatogonia. Glial cell line-derived neurotrophic factor (GDNF) was used as a marker of SC functionality. SC maturation was evaluated by anti-Müllerian hormone (AMH) IHC and immunoenzymatic assay, and androgen receptor (AR) IHC. LC functionality was determined by testosterone levels. A mixed linear regression statistical model was applied.
Main results and the role of chance: Results are presented as a mean for all patients (except for AR expression). Tissue viability was preserved, as demonstrated by the decrease in and stabilization of LDH release, and evolution of ST scoring, with the percentage of well preserved STs out of the total number of STs showing no statistical difference during culture ($p \geq 0.05$). GDNF was expressed until day 139, exhibiting SC functionality. Moreover, a significant reduction in AMH expression and release evidenced SC maturation, further confirmed in P2 by a statistically significant increase in AR. Testosterone concentrations in supernatant increased (to 52 nM) in both culture media, indicating LC functionality. Spermatogonia were present up to day 139, although the ratio between MAGE A4-positive cells and well preserved tubules was significantly reduced over the culture period. Intratubular Ki67 staining increased on day 10, followed by a decrease on day 27 and stabilization. While SC showed a proliferation peak on day 10 and day 16, the proliferation rate of spermatogonia remained stable. No statistical difference was observed between the two culture media.
Limitations, reasons for caution: Our work on AR expression is not conclusive, probably due to age differences, and should therefore be extended to subgroups of different ages. Loss of spermatogonia constitutes another limitation to evaluating full functionality of SSCs, and merits further investigation.
Wider implications of the findings: Our culture system mimicking the peripubertal testicular microenvironment with SC maturation and LC functionality, the first to use human ITT, opens the door to a deeper understanding of niche and culture conditions to obtain sperm from cryostored ITT, with the aim of restoring fertility after gonadotoxic treatments.
Trial registration number:/

O-291 Protective effects of *N*-acetylcysteine (NAC) on human spermatozoa exposed to etoposide

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Study question: Determine the effect of etoposide, a chemotherapeutic agent, on human spermatozoa and the NAC's cytoprotective properties to preserve spermatozoa quality during exposure to etoposide.
Summary answer: Exposure to etoposide induced severe chromatin alterations and DNA fragmentation on human spermatozoa, however, NAC addition protected the genomic integrity of spermatozoa.
What is known already: Male reproduction is adversely affected by chemotherapeutics. Thus far fertility preservation relies on sperm cryopreservation, but most patients fail to collect semen before treatment and pre-pubertal boys do not produce mature spermatozoa. Etoposide is a semi-synthetic chemotherapeutic that induces permanent double-stranded breaks, affecting spermatogenesis and the overall male reproductive capacity. Therefore, there is a need to identify compounds able to preserve fertility during etoposide exposure without interfering with treatment. *N*-acetylcysteine (NAC), is an L-cysteine precursor with known chemopreventive and antioxidant properties. Additionally, it has been reported that NAC improves semen parameters and protects Sertoli cells from damage induced by oxidative-stress.
Study design, size, duration: During 2015 and after patients (mean age of 33.8 ± 1.2 years) had given informed and written consent, 10 human semen samples presenting absence of known pathologies and intake of medicines; normal physical examination, hormonal profiles and karyotypes; semen analysis without agglutination, immature forms, leukocytes and microorganisms, and a sperm concentration > 15×10^6/ml were subjected to the chemotherapeutic etoposide, to NAC and both drugs in combination *in vitro*.
Participants/materials, setting, methods: Human semen samples were obtained from patients performing spermiogram analysis at a private ART clinic. After incubation for 2 h with etoposide (25 µg/ml), NAC (50 µM) and both drugs in combination, sperm parameters (motility, vitality and morphology) were evaluated according to the WHO guidelines. Sperm DNA fragmentation and chromatin condensation were evaluated by TUNEL and Aniline Blue assays, respectively. Oxidative damages were measured and sperm metabolism was studied by proton nuclear magnetic resonance spectroscopy (^{1}H-NMR).
Main results and the role of chance: Our results demonstrate that etoposide exposure induces severe spermatozoa chromatin alterations and DNA fragmentation. Moreover, exposure of human spermatozoa to etoposide does not induce cellular oxidative damages nor glycolytic profile alterations, providing evidence that etoposide directly affects the cells' DNA. NAC's addition to spermatozoa exposed to etoposide preserved sperm chromatin condensation and reduced sperm DNA fragmentation. Suggesting that NAC acts as a cytoprotector agent, shielding human sperm DNA from etoposide-induced damages.
Limitations, reasons for caution: Etoposide exposure induces DNA damages to human spermatozoa, compromising fertility and being transmittable to the next generation. While NAC addition seems promising, additional research to identify an effective therapy that preserves male reproductive functions must be performed. Until then, fertility preservation options must be deliberated, before treatment, to allow parenthood.
Wider implications of the findings: NAC's ability to preserve human spermatozoa DNA damages induced by etoposide may be of clinical relevance as most cancer patients fail to collect semen prior to treatment. NAC addition may preserve spermatozoa integrity after chemotherapy, assuring sperm would have genomic integrity and could be safely used in Medically Assisted Procreation.
Trial registration number: Not applicable.

SELECTED ORAL COMMUNICATIONS

SESSION 74: PCOS

Wednesday 06 July 2016 **Room 101** **14:00–15:15**

O-292 Dysregulation of steroids pathways in granulosa cells may contribute to overexpression of anti-Müllerian hormone system in women with polycystic ovary syndrome

A. Pierre[1], J. Taieb[1], R. Fanchin[1], H. El Hachem[1], M. Grynberg[1], J. Cohen-Tannoudji[1], N. di Clemente[1], C. Racine[1]
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Study question: Is the expression of the anti-Müllerian hormone (AMH) system dysregulated by steroids in granulosa cells (GCs) from women with the polycystic ovary syndrome (PCOS)?
Summary answer: Our results demonstrate a dysregulation of steroids signaling in GCs from PCOS women that can contribute to the overexpression of AMH system.
What is known already: AMH and its specific receptor (AMHRII) are expressed in GCs of growing follicles. AMH exerts a repressive role on folliculogenesis. AMH and AMHRII are overexpressed by GCs of women with the PCOS, the most common cause of female infertility. Previously, we have shown that LH could be one of the factors responsible for this AMH system overexpression, but the influence of local steroids cannot be excluded. Indeed, in healthy ovaries, testosterone has been shown to repress AMH expression and oestradiol (E2) to up- and down-regulate it through the estrogen receptor alpha (ERα) and beta (ERβ) respectively.
Study design, size, duration: Twenty-five control women and sixteen dysovulatory PCOS women undergoing assisted reproduction technology procedures were included between September 2013 and June 2015. The effects of E2 and dehydrotestosterone (DHT), a non-aromatic androgen, on AMH and AMHRII expression were studied in lutein GCs from control and PCOS women. The expression levels of ERβ, ERα and androgen receptor (AR) mRNAs were compared in GCs from both groups. The intrafollicular levels of AMH and testosterone were also measured.
Participants/materials, setting, methods: After ovarian puncture, CGs were isolated from the follicular fluids of each woman through a percoll gradient. GCs were cultured for 48 h with E2 or DHT before RNA extraction. When possible, an aliquot of CGs was used to compare ERa, ERb and AR mRNA levels in control and PCOS women before culture. Accumulation of AMH, AMRII, ERa, ERb and AR mRNA was quantified by real-time PCR. Intrafollicular AMH and testosterone concentrations were measured by Elisa.
Main results and the role of chance: AMH and AMHRII expression in GCs from control women was not affected by DHT treatment whereas it up-regulated AMH mRNA levels in GCs from PCOS women (2.3-fold, $p < 0.01$). This stimulatory effect of DHT was associated with an overexpression of AR in GCs from PCOS women (1.4-fold, $p < 0.05$). E2 down-regulated both AMH and AMHRII expression in GCs from control women (1.4-fold, $p < 0.001$ and 1.8-fold, $p < 0.01$ respectively) but had no effect on the expression of these genes in GC from PCOS women. Interestingly, GCs from PCOS women expressed more of ERa than GCs from control women (2-fold, $p < 0.05$). Furthermore, a positive correlation was observed between the ERa/ERb ratio and the intrafollicular AMH concentration in PCOS women. The absence of effect of E2 in GCs from PCOS women could thus be due to a dysregulation of E2 signaling in these cells.
Limitations, reasons for caution: Lutein GCs poorly express AMH and AMHR-II genes. However, AMH and AMHR-II mRNAs can be reliably quantified by real time PCR. Assisted reproduction technology procedures are the only way to get enough cells to perform RNA expression studies and to compare cells from control and PCOS women.

Wider implications of the findings: Our results demonstrate that the regulation of AMH and AMHRII by DHT and E2 is altered in PCOS GCs, in a way which promotes their overexpression. Because AMH inhibits follicles sensitivity to Follicle Stimulating Hormone, this dysregulation could participate to the follicular arrest and the anovulation observed in PCOS patients.
Trial registration number: 0.

O-293 A novel method to demonstrate that pregnant women with PCOS hyper-expose their fetus to androgens: a possible stepping stone for the developmental theory of PCOS

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Study question: Are neonatal sebum excretion rates, as indicators of *in utero* exposure to excess androgens, greater in infants born to mothers with PCOS compared to those without?
Summary answer: Neonatal sebum excretion, indicating *in utero* exposure to excess androgens, is evident in most infants of mothers with PCOS compared to none from mothers without PCOS.
What is known already: PCOS has a high degree of heritability. The developmental hypothesis for the origin of PCOS suggests that maternal excess androgens may transfer to the fetus and influence imprinting, leading to altered genetic expression in adult life. Studies on pregnant monkeys injected with testosterone, prevalence of tom-boy behaviour of female children and even an increased incidence of autism have all corroborated the influence of hyper-exposure to testosterone *in utero*. Studies in cord bloods have mainly failed to demonstrate increased concentrations of androgens in newborn but the use of cord bloods has inherent problems.
Study design, size, duration: Prospective case control study to discern whether neonatal sebum excretion is greater in female infants of PCOS mothers compared to non-PCOS. Women with known PCOS (all 3 Rotterdam criteria) and non-PCOS controls, with a female fetus, were recruited at 24 weeks pregnancy and serum testosterone estimated. Sebum was measured for 30 and 60 min within 24 h of birth, at 1 week, 4–6 weeks and 6 months after birth in both mother and child.
Participants/materials, setting, methods: Both PCOS and control group women consented to participate at 20 weeks pregnancy, had blood sampled and were next seen during labour at a single University Hospital. Sebum excretion was measured using Sebutape® in mother and child at birth and at regular intervals at home for 6 months after birth. All semi-quantitative sebum excretion estimations were compared between the two groups and correlated with testosterone concentrations during pregnancy.
Main results and the role of chance: So far in this ongoing study, 17 women have completed the 6 month examination period. The PCOS group (n = 10) and the controls (n = 7) were demographically similar. Mean testosterone was 7.2 nmol/L in PCOS mothers and 3.1 nmol/L in controls at 24 weeks pregnancy. At all time frames, the results of sebum excretion at 30 and 60 min were consistent. The sebum excretion of the mothers in both groups was constant from birth throughout 6 months. All babies were born between 37 and 41 weeks gestational age. Six newborns had detectable sebum excretion at birth, all of them in the PCOS mothers group compared to none in the controls (P = 0.01). Only in one was sebum detectable at 4–6 weeks following delivery. These results suggest that women with PCOS could hyper-expose their fetus to androgens *in utero* and that this may be detected using a simple novel test.
Limitations, reasons for caution: So far, relatively small numbers have completed the full 6 month follow-up. Dry/greasy skin, gestation dates, breast feeding could impact on results. Due to the wider implications of this novel method of examining hyper-exposure to androgens *in utero* and results so far, this is presented as a proof of concept study.
Wider implications of the findings: Higher sebum production in babies of PCOS mothers suggests hyper-exposure of the fetus *in utero* to androgens. Confirmation required but according to the developmental theory of PCOS, this simple novel method could potentially be used to predict development of PCOS in adult life and induce research to eliminate symptoms of PCOS.
Trial registration number: NCT 02654548.

O-294 Prevalence of type 2 diabetes (DM2) at first presentation among women with polycystic ovarian syndrome (PCOS) and normal ovulatory controls

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Study question: What is the prevalence of DM2 screened by fasting and 2-h serum glucose levels in a large prospective population of PCOS and control women?
Summary answer: DM2 occurred 6.3% of PCOS and the 2-h oral glucose tolerance test detects a significant number of women who are missed with fasting glucose levels.
What is known already: Between 25 and 40% of women with PCOS are insulin resistant, which puts them at increased risk of DM2, hyperlipidemia, metabolic syndrome, intra-vascular plaque formation and in theory coronary events. DM2 is itself a risk for cardiovascular disease, malignancy and death. Few large studies have been performed prospectively to detect the rate of DM2 in women with PCOS and the relationship to serum androgen levels. This study was performed to see the rate of DM2 in a large prospective North American population of women with PCOS and normal ovulatory controls using both fasting and 2-h oral glucose tolerance test.
Study design, size, duration: An observational cross-sectional analysis from a longitudinal prospective cohort study was held by a University Health Centre at an endocrinology clinic. Enrolment was commenced in 1987 and continues. IRB approval was obtained and subjects signed consents. A total of 1260 participants were recruited. 841 women had PCOS according to the Rotterdam criteria and 419 were ovulatory controls. Even prior to 2003 women with ovulation, clinical or biochemical hyperandrogenism and polycystic ovaries were classified as PCOS.
Participants/materials, setting, methods: Women were screened for congenital adrenal hyperplasia, androgen secreting tumors of the ovary and adrenals. At initial evaluation they underwent a fasting level and 2-h oral glucose tolerance test (OGTT). Age, BMI and serum markers between control and PCOS subjects were compared. Statistical analysis was performed by logistic regression to control for the confounding effects of age, BMI and serum steroid levels. Correlations were also performed.
Main results and the role of chance: Compared to the control group, PCOS participants are younger (p = 0.0001) and heavier (p = 0.0001), and have higher serum androsteinedione (p = 0.0001), testosterone (total, p = 0.0001 and free, p = 0.0001), dehydroepiandrosterone (DHEA) p = 0.0001), dehydroepiandrosterone-sulfate (DHEA-S) (p = 0.0001) and fasting insulin (p = 0.0001) levels.

Among PCOS women, there was a significant correlation between fasting and 2 h serum glucose levels (r = 0.56, p = 0.0001). As expected serum glucose levels at baseline and 2 h correlated significantly with age (p = 0.004, p = 0.0003) and BMI (p = 0.0001, p = 0.0001) respectively. There were significant negative correlations with serum fasting and 2 h glucose levels and serum DHEAS levels (p = 0.03, p = 0.006), as well as 2 h OGTT results and serum estrone (p = 0.01), estradiol (p = 0.003) and baseline DHEA levels (p = 0.05).

The rate of diabetes at first presentation was 6.3% in PCOS women and 8.3% in control subjects. Among PCOS, the detection rate with FG was 2.7% and 5.7% with 2-h OGTT. PCOS had rates of DM based on body mass index (BMI) groupings of <20, 20–24.9, 25–29.9, 30–39.9, ≥40 kg/m^2 of: 2.8, 5.0, 3.1, 8.1 and 35%, respectively. Controls had rates of DM2 based on these BMI groups of: 0, 2.4, 0, 30 and 20%, respectively.
Limitations, reasons for caution: Subject selection bias may have played a role given the recruited control participants may have volunteered in screening of DM2 because they already knew or thought they belong to a population at risk (e.g., family history of diabetes, high BMI).

Wider implications of the findings: There was a strong negative relationship between serum estrogens or adrenal androgens and blood sugar levels. DM2 is a disease of all BMIs in PCOS, becoming common when BMI ≥40 kg/m^2. Among controls it is a disease of BMI ≥30 kg/m^2. This conclusion is from a young population at initial visit.
Trial registration number: This project was started before registration existed. Also it has not been registered yet given it is an observational study, and not interventional.

O-295 Polycystic ovary syndrome (PCOS), hyperandrogenism and overweight/obesity, independently and interactively, increase the risk of metabolic syndrome

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Study question: What is the impact of PCOS, hyperandrogenism and body mass index (BMI) in the development of metabolic syndrome (MetS) or its components in perimenopausal age?
Summary answer: Overweight/obesity, PCOS and hyperandrogenism, independently and interactively, increased risk of MetS. In normal-weight women, PCOS increased risk of dyslipidaemia, but not of other MetS components.
What is known already: Previous studies (mainly cross-sectional) arising from hospital-based populations have reported a cluster of metabolic risk factors and an increased prevalence of MetS in women with PCOS, but the roles of BMI and hyperandrogenism are not clear. Thus, the present study aimed to investigate in a prospective longitudinal population-based cohort the respective roles of PCOS per se, hyperandrogenism, BMI and weight gain on the risk of developing MetS or its individual components [e.g., dyslipidaemia, hypertension or abnormal glucose metabolism (AGM)] by the age of 46.
Study design, size, duration: In a prospective, general population-based follow-up birth cohort (n = 5889), postal questionnaires were sent at ages 14 (95% answered), 31 (81% answered) and 46 (72% answered). Questions about oligoamenorrhea/hirsutism were asked at age 31, and a question about PCO/PCOS diagnosis at age 46. Clinical examination and blood sampling were performed at age 31 in 3115 women, and at age 46 in 3280 women, and oral glucose tolerance test was performed at age 46 in 2780 women.
Participants/materials, setting, methods: Women reporting both oligoamenorrhea + hirsutism at age 31 and/or diagnosis of PCO/PCOS by age 46 were considered as women with PCOS (N = 279). Women without any symptoms at age 31 and without PCO/PCOS diagnosis by age 46 were considered as controls (n = 1577). MetS was defined according to criteria designed for women with PCOS (Rotterdam 2003 PCOS consensus). Pregnant women and current users of hormonal preparations were excluded. Odds ratios (ORs) were calculated by logistic regression analysis.
Main results and the role of chance: MetS (31.2 vs. 16.6%, $p < 0.001$) and its components [hypertension (63.5 vs. 48.5% $p < 0.001$), dyslipidaemia (77.9 vs. 67.5% $p = 0.002$), waist circumference ≥88 cm (57.7 vs. 43.1% $p < 0.001$) and AGM (24.6 vs. 14.2% $p < 0.001$)] were significantly more prevalent in women with PCOS compared with controls.

Normal-weight (BMI <25.0 kg/m^2) women with PCOS had similar prevalence of MetS (3.2% for both groups), hypertension and AGM, but greater prevalence of dyslipidaemia (75.4 vs. 53.7%, $p = 0.001$) compared to normal-weight controls. Overweight/obese (BMI≥25.0 kg/m^2) women with PCOS had a significantly greater prevalence of MetS (46.8 vs. 28.9%, $p < 0.001$), hypertension (72.8 vs. 63.1%, $p = 0.022$) and AGM (33.9 vs. 20.6%, $p = 0.001$), but similar prevalence of dyslipidaemia to overweight/obese controls.

Women with PCOS+MetS had significantly greater BMI at age 14 (p = 0.026) and 31 ($p = 0.002$), weight gain between 31 and 46 years ($p = 0.011$) and free-androgen-index (FAI) at age 46 ($p = 0.026$ after adjustment for BMI) compared with controls with MetS.

In multivariate logistic regression analysis, overweight/obesity, PCOS and hyperandrogenism, independently and interactively, increased risk of MetS. In model I, the OR for overweight/obesity was 13.7 [95% confidence interval (95% CI): 8.54–22.0] and for PCOS 2.02 (95% CI: 1.37–2.98). In model II, the OR for overweight/obesity was 10.30 (95% CI: 7.42–14.32) and for hyperandrogenism 1.75 (95% CI: 1.24–2.46) for the third and 3.14 (95% CI: 2.27–4.34) for the fourth FAI-quartile.
Limitations, reasons for caution: The symptoms and the diagnosis of PCOS were based on self-reporting. The questionnaire at 46 years did not distinguish between polycystic ovaries only in ultrasonography and the syndrome. Ovarian ultrasonography was not available to aid the diagnosis of PCOS.
Wider implications of the findings: These results emphasize the role of early weight management in prevention of MetS and its components in PCOS, as PCOS and overweight seem to increase synergistically the prevalence of MetS. Also normal-weight women with PCOS are at risk for dyslipidaemia, and therefore lipid screening should be considered.
Trial registration number: NA.

O-296 GnRH agonist for triggering final oocyte maturation in patients with PCO at high risk for severe OHSS: factors predicting live birth in subsequent FRET cycles

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Study question: Which are the factors that predict live birth in frozen–thawed (FRET) cycles, following gonadotrophin-releasing hormone (GnRH) agonist triggering in high-risk for OHSS patients with PCO?
Summary answer: Number of 2pn oocytes and male factor infertility positively predict live birth during FRET cycles after GnRH-agonist triggering in high-risk for OHSS patients with PCO.
What is known already: The substitution of human chorionic gonadotrophin (hCG) with GnRH agonist for triggering final oocyte maturation is known to eliminate severe ovarian hyperstimulation syndrome (OHSS), thus, enhancing safety of ovarian stimulation. Cryopreservation of all embryos and postponement of embryo transfer in subsequent FRET cycles has been proposed to manage the decreased probability of pregnancy, following agonist triggering and fresh embryo transfer. However, it is still not known whether there are any factors that can predict live birth in these patients.
Study design, size, duration: This is a prospective, observational study of 150 patients with polycystic ovaries (PCO), according to Rotterdam criteria, in whom GnRH final oocyte maturation was triggered with GnRH agonist and embryo transfer was performed in 227 subsequent FRET cycles (range: 1–4), between September 2011 and December 2015.

Participants/materials, setting, methods: Following stimulation with recombinant gonadotrophin, GnRH antagonist and triggering of final oocyte maturation with GnRH agonist, all resulting 2-pronuclei (2pn) oocytes were cryopreserved. The number of 2pn oocytes thawed in each subsequent FRET cycle varied according to the couple's choice, regarding number and stage of embryos to be transferred. Cumulative live birth was estimated by Kaplan–Meier survival function, while Cox proportional-hazards regression was used to assess the predictive ability of various factors on live birth.

Main results and the role of chance: The mean (95% CI) age of patients included was 31.7 (31.2–32.3) years. On the day of agonist triggering, the mean level of estradiol was 4174 (3913–4436) pg/ml, while the mean number of follicles ≥11 mm developed was 25.2 (24.1–26.3). At oocyte pick-up, 20.4 (19.2–21.7) oocytes were retrieved and following fertilization, 11.4 (10.6–12.2) 2pn-oocytes were cryopreserved. Cumulative live birth rate after up to four FRET cycles (1.8, 95% CI: 1.7–1.9 cycles per patient) was 59.8% (95% CI: 45.6–74.4).

Multivariable analysis showed that the presence of male factor (HR: 1.81, 95% CI: 1.04–3.16, $p = 0.036$) and the number of 2pn oocytes (HR: 1.05, 95% CI: 1.02–1.08, $p = 0.001$) significantly predicted live birth, controlling for the effect of female age, number of embryos thawed, number of embryos transferred and stage of embryos at transfer, variables which were not significant in predicting the probability of live birth in univariable analysis. Controlling for the above variables, the presence of male factor in a couple, relatively increased the risk of live birth by 81% per FET cycle, while every additional 2pn oocyte resulted in a relative increase in the risk of live birth by 5% per FET cycle.

Limitations, reasons for caution: A larger study would be necessary to increase the precision of the estimates derived from Cox regression analysis.

Wider implications of the findings: The findings of this study might help clinicians counsel patients with PCO, at high risk for OHSS, regarding the probability of live birth and the presence of relevant predictive factors after GnRH agonist triggering, cryopreservation of all embryos and embryo transfer in subsequent FRET cycles.

Trial registration number: –

Posters Presentations

POSTER VIEWING SESSION

ANDROLOGY

P-001 Anti-oestrogen administration increases pregnancy rates in patients with idiopathic male subfertility: a systematic review and meta-analysis

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Study question: Is the use of anti-estrogens associated with a higher probability of pregnancy in patients with idiopathic male subfertility?
Summary answer: The use of anti-oestrogens in patients with idiopathic male subfertility is associated with a higher probability of pregnancy as compared to no treatment/placebo.
What is known already: Idiopathic oligoteratoasthenospermia (OTA) represents a challenging clinical condition for the andrologist and several treatment alternatives have been proposed in the published literature. These include anti-oestrogens, anti-oxidants, various forms of androgens, as well as combinations of the above. Anti-oestrogens (tamoxifene and clomiphene citrate) have been used extensively, although there is conflicting data on whether their administration is associated with an improvement in semen characteristics and most importantly in the probability of pregnancy.
Study design, size, duration: This is a systematic review and meta-analysis of randomized controlled trials in which the administration of anti-estrogens was compared to placebo or no treatment in patients with idiopathic male subfertility. Following the development of a search strategy, a computerized literature search was performed in the MEDLINE, EMBASE, CENTRAL, Scopus and Web of Science aiming to identify eligible studies published until 8/1/2016.
Participants/materials, setting, methods: Eligible studies were those offering comparative data from parallel randomized studies. Retrieved citations were screened by two independent reviewers. Primary outcome measure was the achievement of pregnancy and secondary outcome measures included semen analysis characteristics. The effect of anti-oestrogen administration on the hormonal profile (FSH, LH, testosterone) was also evaluated. Standard meta-analytic methodology was used to statistically synthesize the results while the quality of the retrieved studies was also assessed.
Main results and the role of chance: Eleven RCTs, published between 1983–2015, were identified (study median size: 70 patients). Most studies compared tamoxifen with placebo/no treatment (n = 7), while four RCTs studies compared clomiphene citrate with placebo/no treatment. The quality of the included RCTs was generally considered moderate-to-low.

Administration of anti-oestrogens for 3 months resulted in an increase in serum testosterone concentration (standardized mean difference-SMD:+0.69, 95% CI:+0.39 to +0.98) and serum FSH (SMD:+0.80, 95% CI:+0.50 to +1.10) as compared to placebo/no treatment. Serum LH was not significantly different between the anti-oestrogen and control group at 3 months (SMD:+0.46, 95% CI:−0.61 to +1.53).

Sperm count was increased in patients who received anti-oestrogens as compared to those who did not both at 3 (SMD:+0.34, 95% CI:+0.11 to +0.57) and 6 months (SMD:+1.56, 95% CI:+1.25 to +1.88) of treatment. Sperm motility was not significantly increased at 3 months (SMD:+0.24, 95% CI:−0.02 to +0.51) but it appeared significantly higher at 6 months (SMD:+0.86, 95% CI:+0.50 to +1.22). Sperm morphology at three months was not associated with the administration or not of anti-oestrogens.

The probability of spontaneous pregnancy at six months of clomiphene or tamoxifene administration was significantly higher than in the placebo/no treatment group (OR: 1.73, 95% CI:1.03–2.92, n = 531 patients).

Limitations, reasons for caution: Most of the studies included are relatively small and of moderate-to-low quality. Furthermore, heterogeneity between those studies was not negligible and should be taken into account when interpreting the results of this meta-analysis.
Wider implications of the findings: Available evidence suggests that the administration of anti-oestrogens in subfertile men with idiopathic OTA might increase the probability of spontaneous conception. Moreover, it might improve sperm count and motility potentially rendering the provision of intrauterine insemination a more attractive option. Further relevant RCTs are warranted.
Trial registration number: None.

P-002 Levels of reactive oxygen species (ROS) in the seminal plasma predicts the effectiveness of L-carnetine to improve sperm function in men with infertility

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Study question: To investigate whether L-carnetine significantly improves sperm function, and whether baseline levels of seminal plasma ROS predicts its effectiveness.
Summary answer: 3 month course of L-carnetine (Proxeed Plus) therapy significantly increased total motile sperm count (TMC) in patients with oligoaesthenospermia and elevated baseline levels of ROS,
What is known already: Anti-oxidants such as L-carnetine are commonly used to treat male infertility. Some previous reports suggest L-carnetine improves sperm quality in men, but its mechanism of action is currently unclear. No previous study has investigated whether the anti-oxidant effects of L-carnetine relate to changes in sperm quality, in men with infertility.
Study design, size, duration: Outcome data from a single specialist male fertility clinic were analysed retrospectively. All subjects were treated with Proxeed Plus for ninety days.
Participants/materials, setting, methods: Semen analysis and ROS levels were measured immediately before and following Proxeed Plus treatment. All patients (n = 29) fulfilled the following criteria: infertility >18 months; no previous reported anti-oxidant therapy; no varicocele or intrascrotal pathology. ROS measured in relative light units per second (RLU/s) using an established chemiluminescence assay.
Main results and the role of chance: In men with oligoaesthenospermia (n = 29), L-carnetine reduced ROS markedly (ROS in RLU/s: 105 ± 83, pre-treatment; 6.6 ± 1.8, post-treatment, $P < 0.05$ vs. pre-treatment). Overall, sperm total motile count (TMC) did not change significantly following L-carnetine therapy in men with oligoaesthenospermia (TMC in millions: 24.7 ± 5.7, pre-treatment; 30.4 ± 9.9, post-treatment, $P = 0.37$ vs. pre-treatment). However in men with oligoaesthenospermia and a pre-treatment ROS >10RLU/s (n = 12), L-carnetine increased sperm TMC more than 2-fold (sperm TMC in millions: 18.6 ± 8.8, pre-treatment; 51.2 ± 27.0, post-treatment, $P < 0.01$ vs. pre-treatment).
Limitations, reasons for caution: An RCT is required to confirm the novel observations of this pilot study.
Wider implications of the findings: Men with infertility are increasingly prescribed anti-oxidants, but there remains a paucity of data investigating their effectiveness. We suggest patients with oligoaesthenospermia and elevated levels of ROS may benefit clinically from L-carnetine therapy. These data have important potential implications which warrant further clinical investigation.
Trial registration number: no required.

P-003 Cryopreservation of spermatozoa by means of small and large volume vitrification compared with conventional slow freezing and the effects on post-thaw sperm quality

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Study question: This study aimed to compare two methods of cryoprotectant-free vitrification, i.e., large volume vitrification and small volume vitrification, with conventional slow freezing.
Summary answer: Compared with conventional slow freezing, small volume vitrification resulted in a significantly higher percentage of post-thaw progressive sperm motility.
What is known already: Due to the rapid cooling rates, vitrification generally requires high concentrations of permeable cryoprotective agents (CPA), such as glycerol or dimethylsulfoxide (DMSO), in order to avoid the formation of damaging ice crystals. While this has proven to be successful for the vitrification of human oocytes or embryo's, this method is inappropriate for the cryopreservation of human spermatozoa because of their low tolerance to permeable CPA. Cryoprotectant-free vitrification, whereby a mixture of human serum albumin (HSA) and sucrose is used, could provide a solution to this problem.
Study design, size, duration: The study was designed as an auto-controlled split-sample study. In total, 41 semen samples were collected from men visiting the fertility centre for a routine sperm analysis. After routine sperm analysis and density gradient centrifugation (DGC) with Pure Sperm, the remainder capacitated sperm samples were divided into three aliquots and cryopreserved by conventional slow freezing, large volume (400 µl) vitrification and small volume (40 µl) vitrification respectively.
Participants/materials, setting, methods: Inclusion criteria were based on WHO reference limits for a normal semen sample: healthy viscosity, pH between 7.2–7.8, concentration of ≥15 million/ml with a good forward progressive motility of ≥32% and ≥4% normal morphology. Sperm quality parameters were analysed after DGC prior to freezing and after thawing of the spermatozoa. The routine parameters sperm motility and morphology were taken into account, as well as acrosome reaction, apoptosis and dead spermatozoa analysed by flow cytometry.
Main results and the role of chance: All three cryopreservation methods resulted in a significant reduction of progressive sperm motility and normal morphology and an increased percentage of acrosome reacted, apoptotic and dead spermatozoa compared with the DGC prepared unfrozen sperm sample ($p < 0.05$). Cryopreservation using large volume vitrification generated a significantly lower post-thaw progressive sperm motility (17.05 ± 1.19 vs. 22.12 ± 1.13; $p < 0.0001$) and higher percentages of acrosome reacted (26.70 ± 3.19 vs. 20.23 ± 2.42; $p = 0.0003$) and dead spermatozoa (69.53 ± 3.45 vs. 60.79 ± 3.55; $p = 0.007$) compared with conventional slow freezing. Small volume vitrification on the other hand, showed a significantly higher post-thaw progressive sperm motility (24.68 ± 1.48 vs. 22.12 ± 1.13; $p = 0.0244$) compared with conventional slow freezing, but also significantly increased the percentage of acrosome reacted spermatozoa (27.39 ± 3.45 vs. 20.23 ± 2.42; $p = 0.0028$). When comparing small and large volume vitrification, small volume vitrification resulted in a significantly higher post-thaw progressive sperm motility (24.68 ± 1.48 vs. 17.05 ± 1.19, $p < 0.0001$) and significantly lower percentages of apoptotic (8.27 ± 1.13 vs. 11.42 ± 0.76; $p = 0.0011$) and dead spermatozoa (69.44 ± 2.114 vs. 69.53 ± 3.45; $p = 0.0266$).
Limitations, reasons for caution: The amount of DNA damage was not taken into account as a parameter of sperm quality. Furthermore, vitrification of spermatozoa has only been researched *in vitro*. There are no results indicating fertilisation and pregnancy rates or birth numbers of healthy new-borns.
Wider implications of the findings: With further research, vitrification of spermatozoa could provide interesting options for future IVF lab practices, as it has previously been described as a simpler, faster and more cost-effective technique than conventional slow freezing.
Trial registration number: Not applicable.

P-004 Determining the prevalence of human papillomavirus in sperm donors

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Study question: Which is the HPV prevalence in sperm donors?
Summary answer: The presence of HPV in donor semen poses risks for women taking part in a programme of assisted reproduction with donor sperm.
What is known already: Among both men and women, human papillomavirus (HPV) is one of the pathogens that is most commonly transmitted through sexual relations. Over 100 serotypes have been identified, of which about 40 infect the genital tract. Studies have reported reduced rates of fertilisation and pregnancy and higher rates of miscarriage when semen samples with HPV are used in *in vitro* fertilisation and in intracytoplasmic sperm injection.
Study design, size, duration: 678 sperm ejaculates corresponding to 210 sperm donors were cryopreserved between 2010 and 2014. After thawing HPV was determined. When a positive HPV result was obtained the amplified product was then classified by sequencing.
Participants/materials, setting, methods: 435 ejaculates were cryopreserved after direct addition of cryoprotectant medium to the ejaculate. 243 ejaculates were cryopreserved after selection of the motile sperm according to density gradients, after which cryoprotective medium was added. Computerised system was used for subsequent freezing. Samples were thawed following a rapid thawing protocol at room temperature for ten minutes. The presence of HPV was determined by conventional PCR. The amplified product was detected by agarose electrophoresis and ethidium bromide staining.
Main results and the role of chance: None of the donors presented clinical symptoms of HPV infection at the time of sperm donation. Of the 210 donors studied, the PCR for HPV was negative in 206 cases (98%), non-quantifiable in 3 (1.5%) and positive in 1 (0.5%), in which the presence of DNA from HPV type 66 was detected.
Limitations, reasons for caution: The presence of HPV in donor semen poses risks for women taking part in a programme of assisted reproduction with donor sperm. Therefore, the detection of HPV in donor semen by screening for sexually transmitted diseases during donor selection would enhance patient safety.
Wider implications of the findings: The low prevalence observed (0.5%) may be due to stringent values for donation (concentration >80 × 106 spermatozoids/mL; full motility >50%; morphology >15%). Researchers have shown that the presence of HPV in semen samples is often associated with alterations to sperm parameters such as volume, viscosity, pH, concentration, motility and vitality.
Trial registration number: No.

P-005 Lamivudine, a reverse transcriptase inhibitor, impairs sperm parameters in mice

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Study question: To address the effect of Lamivudine, a reverse transcriptase inhibitor, on sperm parameters of FVB/N mice.
Summary answer: Lamivudine caused a significant reduction on the sperm concentration and on percentage of progressively motile spermatozoa. Increased morphological anomalies, especially in midpiece, were also observed.
What is known already: Reverse transcriptase inhibitors have been used in HIV and Hepatitis patients. Several studies have reported a significant effect of antiretroviral drugs on the sperm parameters of HIV-infected patients causing a decrease in the ejaculate volume, the percentage of motile spermatozoa, the sperm motility and in the normal sperm morphology.
Study design, size, duration: Immature male FVB/N mouse 21 days old, were treated with the nucleoside analog reverse transcriptase inhibitor Lamivudine in a dose of 50 mg/kg, for 7 weeks. After treatment, sperm parameters were examined.
Participants/materials, setting, methods: Male mice were sacrificed by cervical dislocation and sperm was collected from cauda epididymis. Sperm concentration and morphological anomalies were determined using a Neubauer hemocytometer. The motility was classified in rapid progressive motility, slow progressive motility, non-progressive motility and no motility.

Main results and the role of chance: The administration of the reverse transcriptase inhibitor Lamivudine impaired the sperm characteristics of male FVB/N mice. The concentration of spermatozoa was markedly reduced in Lamivudine treated mice compared to control mice. The percentage of rapid progressively motile spermatozoa was also decreased and the majority of spermatozoa were moved slowly in circles. Increased morphological anomalies especially in the midpiece and neck defects were also observed. We conclude that the reverse transcriptase inhibition impairs the sperm parameters in mice possibly through the disruption of normal cell growth and differentiation.
Limitations, reasons for caution: Nucleoside analog reverse transcriptase inhibitors may act against reverse transcriptase but also through other pathways such as inhibition of mitochondrial DNA polymerase. However, the exact mechanism of Lamivudine action needs to be further elucidated.
Wider implications of the findings: The results of present study indicate a negative effect of Lamivudine on sperm parameters and potentially on reproductive outcomes. Lamivudine is used as an antiretroviral drug in HIV and Hepatitis patients. Men undergoing such treatment should be regarded as a high risk infertility group in need for a specialized approach.
Trial registration number: No clinical trial.

P-006 A new simple and quick method for sperm preparation and selection prior to ICSI procedure

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Study question: The aim of this study is to validate new sperm preparation technique using as reference the standard ones. The primary outcome is clinical pregnancy rate.
Summary answer: The results show that our sperm preparation method for ICSI is not inferior compared to traditional techniques, involving several benefits.
What is known already: Semen preparation procedures before ICSI help either to select the best spermatozoa in terms of motility and morphology, or to separate ROS (Reactive Oxygen Species) through washing and centrifugation. Actually in IVF laboratories the most used are swim-up and gradient centrifugation. During these procedures, the sample is centrifuged and passed from a tube to another many times in a period of time that ranges from 45 to 60 min for swim-up to 60 to 90 min for gradient centrifugation making the process time-consuming.
Study design, size, duration: This is retrospective cohort study including 460 couples treated in our Centre between January 2013 and December 2104. Inclusion criteria were: at least 2 millions motile spermatozoa, female age <38 years, no previous pregnancy, no PCOD, no endometriosis, normal AMH, and at least 4 MII oocytes retrieved. The patients were divided in two similar groups: Group A underwent the new technique was used ($N = 230$) and Group B underwent the standards preparation techniques were used ($N = 230$).
Participants/materials, setting, methods: The new preparation technique consist in the preparation of the ICSI plate with three big drops of medium. Depending on it's concentration, 1 to 3 microliters of it were injected in the proximal drop some minutes before adding the oocytes. We were able to select motile spermatozoa also in case of hyper viscous sperm samples, simply using a micropipette to take a very small amount of sample. No additional procedures were necessary.
Main results and the role of chance: At the moment of ICSI, an adequate number of spermatozoa (>20) reached the superior edge of the distal drop in 100% of cases. De facto we performed an "horizontal layering swim-up", removing spermatozoa from the oxidant environment of the seminal plasma. The final dilution of the semen in the clean buffered culture medium was from 1:60 to 1:20.

The clinical pregnancy rates obtained in the 2 groups were similar: 87/230 (37.7%) in Group A and 84/230 (36.5%) in Group B. Laboratory outcomes were al similar in terms fertilization rate, cleavage rate and blastocysts formation. Moreover, none of the cases developed bacterial infection during handling.
Limitations, reasons for caution: It remains to evaluate if our method can also be beneficial to reduce spermatozoa stress during *in vitro* manipulation.
Wider implications of the findings: The results show that our method for ICSI is not inferior compared to traditional techniques.

However compared to the traditional method, our undoubtley reduces strongly the time need for semen preparation with a lower risk of samples mix-up. Unnecessary semen centrifugation may also be beneficial for the spermatozoa avoiding mechanical stress.
Trial registration number: no trial number for this work.

P-007 Characterization of sperm DNA quality in men presenting with reproductive and non-reproductive cancers

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Study question: Do men presenting with cancer have different types of sperm DNA damage, dependant on disease origin?
Summary answer: Men with both types of cancer had higher levels of both single stranded (SS) and double stranded (DS) DNA damage compared with healthy fertile donors.
What is known already: The presence of SS, but not DS sperm DNA damage has been reported in cancer patients. Further, since different assays were employed to measure sperm DNA damage, the proportion of damage is conflicting. In this study, we compared the levels of DS and SS DNA breaks in the same semen samples using three different DNA damage assays: alkaline and neutral Comet and TUNEL in men presenting with testicular cancer and haematological malignancies in comparison with fertile donors.
Study design, size, duration: Men presenting with testicular cancer ($n = 19$) and haematological malignancy ($n = 13$) at the Andrology Unit, Department of Experimental and Clinical Biomedical Sciences, University of Florence between 2014 to 2015 were recruited into the study. The project was approved by the local research ethics and clinical governance committees and written informed consent for participation was obtained from each subject.
Participants/materials, setting, methods: Semen samples were obtained after 3–7 days of sexual abstinence. All semen samples were analysed according to World Health Organisation guidelines (WHO 2010). Semen samples were used immediately for analysis for DNA damage by the TUNEL assay, and an aliquot were cryopreserved for the later analysis of DNA damage by both Comet assays. Comparisons of DNA damage and seminal parameters between cancer patients and fertile donors were assessed using Mann-Whitney U test ($p < 0.05$).
Main results and the role of chance: The mean values of semen parameters of the two patient groups were above the normal ranges of the WHO standards, 2010. Sperm DNA damage was significantly higher in testicular cancer patients; both in terms of DS breaks (13.4% vs. 7.5%; $p < 0.05$) and in the more abundant SS breaks (37.4% vs. 12.4%; $p < 0.001$) than in the fertile donor group. Similar results were obtained in the sperm of men with haematological malignancy. Here, the average amount of single strand breaks per sperm was 35.0% compared with 12.4% in the donor group; ($p < 0.001$), and there was 10.7% of DS damage per sperm against 7.0% in donor sperm ($p < 0.001$). In contrast, there was no significant difference in sperm DNA damage between patient [(25.6% vs. 27.9% for testicular cancer, and 27.7% vs. 27.9% for haematological malignancies; $p>0.05$)] and donor groups when measured with the TUNEL assay.
Limitations, reasons for caution: The study was limited by the limited number of samples included in each group. Also, as the sperm concentration was inadequate for the performance of all assays on every subject's sample, some samples were only included in two of the three assays.
Wider implications of the findings: The novelty of this study lies in comparing DS with SS DNA damage and test sensitivity in men with two types of cancer and ascertaining sensitivities. Longitudinal studies are ongoing to investigate the inducibility and reversibility of SS and DS breaks following cytotoxic therapy.
Trial registration number: N/A.

P-008 Total motile sperm count has a superior predictive value over the WHO 2010 cut-off values for the outcomes of intracytoplasmic sperm injection cycles

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Study question: Does the total motile sperm count (TMSC) have a superior predictive value for the ICSI outcomes over the World Health Organization (WHO) cut-off values?
Summary answer: In couples with male infertility, the TMSC has a superior predictive value over the WHO 2010 cut-off values for laboratory and pregnancy outcomes after ICSI.
What is known already: Semen analysis is recommended for the investigation of sperm quality and relies on cut-off values that were defined by the WHO in 2010 to distinguish between normal and abnormal samples. However, several reports suggest that the correlation between the WHO classification system and the probability of natural or assisted conception is minimal, if any. Individual semen parameters like volume, concentration, and motility can be combined, resulting in an alternative way to express sperm quality (TMSC), which is obtained by multiplying the volume of the ejaculate by the sperm concentration and the proportion of progressive motile sperm divided by 100%.
Study design, size, duration: This prospective study included patients undergoing ICSI from December 2012 to April 2014 at a private fertility centre. Inclusion criteria were: couples undergoing their first ICSI cycle with fresh embryo transfer performed on day 5 of development, as a result of male infertility as per the WHO 2010 classification system. Three individual statistical analyses were performed.
Participants/materials, setting, methods: Couples were divided into: group-I, TMSC $<1 \times 10^6$; group-II, $1–5 \times 10^6$; group-III, $5–10 \times 10^6$; group-IV, $10–20 \times 10^6$; and group-V, $>20 \times 10^6$, the latter being considered a normal TMSC value. Groups I-IV were combined to form the abnormal TMSC group, and the ICSI outcomes in this group and the normal TMSC group were compared. Finally, the influence of the WHO cut-off value and TMSC on ICSI outcomes was investigated.
Main results and the role of chance: A total of 518 ICSI cycles were analysed. All 518 men were diagnosed with male factor infertility according to the WHO classification system. Conversely, when TMSC was used, 36.7% had abnormal sperm and 63.3% were normal. According to the TMSC, 26 couples were in group-I, 50 in group-II, 38 in group-III, 76 in group-IV, and 328 in group-V. The fertilization rate was significantly lower in group-I compared to group-V (72.5 ± 17.6 vs. 84.9 ± 14.4). The normal TMSC group demonstrated significantly higher fertilization (84.9 ± 14.4 vs. 81.1 ± 15.8) and lower miscarriage (17.9% vs. 29.5%) rates than the abnormal TMSC group. The fertilisation rate was influenced by the sperm concentration (RC: 3.994, R^2: 1.4%), morphology (RC: 8.735, R^2: 0.9%) and TMSC (RC: 3.784, R^2: 1.5%). The formation of high-quality zygotes was influenced by the sperm concentration (OR: 1.64, CI: 1.09–2.46) and TMSC (OR: 1.13, CI: 1.01–1.28). The TMSC was the only parameter to influence the formation of high-quality embryos on D2 (OR: 1.18, CI: 1.03–1.35) and D3 (OR: 1.12, CI: 1.07–1.29), the formation of blastocysts (OR: 1.16, CI: 1.04–1.26), blastocyst expansion (OR: 1.27, CI: 1.01–1.60), and the odds of miscarriage (OR: 0.52, CI: 0.28–0.90).
Limitations, reasons for caution: In this study, a TMSC $> 20 \times 10^6$ was considered normal. Even though previous studies have used this cut-off value, there is no consensus on the normal value. Moreover, an alteration to any seminal parameter, as per the WHO 2010 guidelines, was considered as male factor infertility.
Wider implications of the findings: This is the first study to investigate the association between the TMSC and ICSI outcomes. As these are novel findings for infertile patients undergoing ICSI treatment, prospective randomized studies should be performed to investigate whether the TMSC grading is superior to the WHO classification system for classifying male infertility.
Trial registration number: N/A.

P-009 Sperm DNA integrity test and ART outcome

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Study question: To investigate influence of sperm DNA fragmentation index (DFI) on pregnancy outcome and pregnancy loss after ART procedure (autologus ICSI, donation eggs ICSI) and IUI.
Summary answer: The three group categories showed that samples with DFI>27% were associated with increased risk of early pregnancy loss.
What is known already: Results from assessment of sperm DNA fragmentation [DFI] by analyzing sperm chromatin structure (DNA Integrity test), have impact on both natural pregnancy and that achieved through ART.

Pilot studies have shown that high levels of DNA fragmentation [DFI>27%] decrease fertility potential in patients undergoing ART procedure, even in men with completely normal standard sperm parameters Therefore, the lack of correlation between conventional parameters of sperm and DNA fragmentation determine DNA fragmentation as a potential source of male infertility in normozoospermic men. That is why further evaluation of sperm DNA fragmentation is required in the study of male infertility.
Study design, size, duration: A prospective study.
Participants/materials, setting, methods: Patients: We investigated men from 531 couples undergoing autologous ICSI procedure [$n = 416$], from couples undergoing donation eggs procedure [$n = 39$] and IUI [$n = 76$].

Interventions: semen analysis, DNA integrity test, embryo scoring by Gardner and Schoolcraft grading system [1999].
Main results and the role of chance: The study shows no statistically significant differences between the group regarding pregnancy rate. [$\chi^2 = 0.55$. $p > 0.05$, OR = 1.25]. However, with increased levels of DFI, the number of pregnancy losses became higher [including biochemical pregnancies and spontaneous abortions] at OR = 5.65. We examined the percentage of grade I blastocysts [by Gardner and Schoolcraft] before donation eggs embryo transfer and found a statistically significant correlation with both the DFI [$\chi^2 = 7.80$, $p < 0.05$] and sperm morphology [$\chi^2 = 6.14$; $p < 0.05$]. Analysis of the relationship between DFI and IUI output (clinical pregnancy, miscarriage) revealed significant correlations in both directions: between DFI and pregnancy rate after IUI ($\chi^2 = 6.29$, $p < 0.05$) and between the DFI and pregnancy development after IUI ($\chi^2 = 6.87$; $p < 0.05$).
Limitations, reasons for caution: No limitation.
Wider implications of the findings: Men with infertility should undergo DNA fragmentation assay in addition to the standard semen analysis. When DFI exceeds 27%, ICSI should be a method of choice, even in cases where the conventional parameters of semen analysis tests are normal.
Trial registration number: NO.

P-010 In male infertility folate cycle genes variants associate with sperm aneuploidy and DNA fragmentation

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Study question: Do the polymorphic variants of genes MTHFR (C677T, A1298C) and MTRR (A66G) associate with sperm quality in men with low reproductive function?
Summary answer: Polymorphic alleles in genes of folate metabolism are associated with sperm aneuploidy. The correlation between the genotypes and sperm DNA fragmentation is proved.
What is known already: Polymorphic variants of individual genes controlling stages of spermatogenesis may be associated with motility, morphology and fertility properties of sperm. A decrease in the functional activity of enzymes of folate metabolism is discussed as possible reason of failures in formation of the mature male gametes. Spermatogenesis failures in different stages can lead to DNA damage and chromosome abnormalities in sperm nuclei.
Study design, size, duration: Totally the DNA samples of 136 infertile men with mean age 37.3 ± 5.9 years old were used to study the genotypes of genes MTHFR (C677T, A1298C) and MTRR (A66G). Group I consisted of 112 men: a level of sperm DNA fragmentation was determined for patients in Group I. Group II consisted of 24 men: the level of aneuploid sperm in the ejaculate was determined in Group II. Study's protocol was approved by IRB.
Participants/materials, setting, methods: Real-time PCR was done with the ABI PRISM 7500 real-time PCR system (USA), and SNPs determinations in

the MTHFR and MTRR genes were performed with Applied BioSystem kits (USA). Sperm DNA fragmentation analysis was performed using the method of sperm chromatin dispersion (SCD). The sperm aneuploidies in chromosomes 13, 16, 18, 21, X any Y were analyzed exploiting the method of fluorescence in situ hybridization (FISH) using DNA probes Vysis-Abbott (USA).

Main results and the role of chance: The association of the genotype frequencies of polymorphic variants A66G in MTRR with high level of DNA fragmentation in sperm is proved (df = 2, χ^2 actual = 37.95, χ^2 critical = 9.21, $P < 0.01$). It was found that homozygotes CCAA, TTAA, CCCC for single nucleotide polymorphisms C677T and A1298C in MTHFR gene the level of aneuploidy in sperm for chromosome 16 was significantly higher than in heterozygotes CTAC, CTAA, CCAC (Uempiric = 0, Ucritical = 0, $P \leq 0.05$). For heterozygotes A66G in gene MTRR the average level of aneuploidy in sperm is significantly lower than homozygotes A66A (Uempiric = 1.0 Ucritical = 4.0 $P < 0.05$) for all analyzed chromosomes. A positive correlation between the number of polymorphic alleles of single nucleotide polymorphism A1298C of the MTHFR gene and the level of aneuploidy for chromosome 16 in patients younger then 35 years old (rs = 0.86 ± 0.21, Rcritical = 0.72, $P < 0.05$) is found out.

Limitations, reasons for caution: Present technique can evaluate the DNA fragmentation and aneuploidy level in cases, when the sperm count excides 2 mln/mL.

Wider implications of the findings: The aneuploid sperm or sperm with damaged DNA is able to fertilize the oocytes, but the further formation of the blastocyst and embryo implantation may be blocked at various stages of development. Understanding the genetic basis of aneuploidy in sperm of men will lower the reproductive losses in ART.

Trial registration number: No registration number.

P-011 The novel device of male infertility screening with single-ball lens microscope and smartphone

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Study question: What is a new method to screen male infertility at home?

Summary answer: We developed an novel semen analysis device consisting of a single-ball lens microscope paired with a state-of-the-art smartphone equipped with a camera.

What is known already: Semen analysis is the key element in the diagnosing the reproductive potential of a male subject. In current practice men must use a clinic or other hospital facility to have their semen analyzed. Once at the clinic, they are asked to produce a semen sample, which is then analyzed by staff at the clinic. Many subjects are not comfortable with this procedure, which they often find embarrassing and expensive.

Study design, size, duration: This study is a laboratory investigation performed in a single university hospital from November 2015 to January 2016.

Participants/materials, setting, methods: We developed a Leeuwenhoek's microscope constructed with a single-ball lens of 0.8mm in diameter inserted into a plastic jacket that attaches to commercial smart phone. The magnification provided by this ball lens was 555 times. A total of 35 semen samples obtained from volunteers were analyzed with a ball lens and three types of smartphone. We analyzed semen samples both with our device and CASA software.

Main results and the role of chance: Sperm concentration counted with a ball lens and each smartphone showed a very strong correlation with the CASA results ($P < 0.001$). In addition, Sperm motility calculated with our device showed significant correlations to CASA ($P < 0.001$). If we found 8 spermatozoa or less on the field of view of iPhone 6s; 5 spermatozoa or less on the field of view of iPhone 5s and LG Optimus, the semen specimens were considered to below the lower reference limit for sperm concentration of WHO 2010 (15 million/ml spermatozoa). The sensitivity was 87.5% and specificity was 90.4% with iPhone 6s. The sensitivity was 100% and specificity was 95.2% with iPhone 5s. The sensitivity was 100% and specificity was 90.4% with LG Optimus Exceed 2.

Limitations, reasons for caution: A disadvantage of ball lens device are the pincushion and reduction in resolution at the periphery of the field of view, which results in only small region of the capture image being in focus due to spherical nature of the focal surface.

Wider implications of the findings: Smartphones have great potential to support semen analysis because they are portable and contain excellent digital cameras. Single-ball lens microscope is inexpensive and easy to use for acquiring digital microscopic movies. This single-ball lens microscope provides an easy solution for global users to screen male infertility at home.

Trial registration number: None.

P-012 Seminal fluid static oxidation reduction potential is lower in seminal fluid that meets WHO criteria for fertile men

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Study question: Identify a cut-off value for sORP that can be used to identify semen that meet the normal reference range of WHO parameters from ones that fail.

Summary answer: Over 90% of samples with a sORP <1.635 mV/sperm concentration were correctly identified as meeting all WHO parameters.

What is known already: Discrete measures of free radicals, antioxidant activity, and oxidative damage suggest an ambiguous relationship between the redox system and male fertility. sORP measures the balance between all oxidants and antioxidants providing a comprehensive status of the redox system. In previous studies, sORP was elevated in brain trauma, stroke, and multitrauma patients, indicating a state of oxidative stress; thus sORP levels may be used to clarify the relationship between the redox system and semen parameters associated with male infertility. Further, a sORP cut-off value may identify semen samples that meet the WHO normal reference values.

Study design, size, duration: This prospective study was carried out in the Male Infertility Clinic of Hamad Medical Corporation on 400 subjects. The study was approved by the institutional review board and patients signed a consent prior to participation. The study subjects were grouped into those that had all normal semen parameters by WHO 2010 guidelines (Group A) and those who failed to meet one or more criteria (Group B).

Participants/materials, setting, methods: Exclusion criteria included azoospermia, presence of STD or chronic disease, use of prescription or OTC medications or antioxidants. Samples were collected and semen parameters assessed using the WHO guidelines (5th edition, 2010). Parameters included: volume, total number, concentration, total motility, progressive motility, and morphology. sORP was measured (mV) using the MiOXSYS system and normalized to concentration (mV/10^6 sperm/mL). For group comparisons, only those samples with a concentration >0.999 × 10^6 sperm/mL were included.

Main results and the role of chance: 364 samples failed to meet one or more criteria for sperm quality; 36 met all WHO criteria. sORP values were significantly lower in samples from Group A ($p < 0.05$). A cut-off value of >1.635 mv/10^6 sperm/mL correctly identified 91.43% of those that met all WHO criteria. Majority of samples (96%) with four or more abnormal sperm parameters ($n = 135$) were identified by the cut-off value. With an increasing number of failed parameters, sORP values also increased suggesting an elevating state of oxidative stress with increasing abnormality. The most commonly failed parameter was progressive motility (97.3%) but this alone did not significantly contribute to increased sORP values. Morphology was the next frequently abnormal parameter (59.0%). Controlling for other criteria, morphology significantly increased sORP, suggesting that oxidative stress is associated with abnormal morphology ($p < 0.05$). The sORP cut-off value (>1.635 mV/sperm concentration), while excellent at differentiating semen with multiple abnormal parameters, was less useful in identifying those samples with only a singular abnormality or marginal infertility.

Limitations, reasons for caution: The current study was performed out of a single fertility clinic and since semen parameters as well as the redox system are subject to many lifestyle variables (ie. regional diets and lifestyles) it would be beneficial to explore the sORP values in regards to semen quality using culturally diverse populations.

Wider implications of the findings: sORP levels may be used as an indicator of sperm quality. Patients with high sORP levels in their semen are good

candidates for treatment of underlying pathology (varicocele, infection, etc.) and may benefit by antioxidant supplementation.
Trial registration number: Not a clinical trail.

P-013 Oxidation reduction potential: a new predictor for sperm morphology in infertile men

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Study question: To measure the ORP in semen samples of infertile men and assess its predictive value for abnormal sperm morphology.
Summary answer: ORP level higher than 3.29 mV/106 sperm/ml has an 85.7% chance of having abnormal morphology.
What is known already: Oxidative stress has been associated with infertility in men, and specifically with morphology. sORP measures the balance between all oxidants and antioxidants providing a comprehensive status of the redox system. In previous studies, sORP was elevated in brain trauma, stroke, and multitrauma patients, indicating a state of oxidative stress; thus sORP levels may be used to clarify the relationship between the redox system and semen parameters associated with male infertility.
Study design, size, duration: This prospective study was carried out in the Male Infertility Clinic of Hamad Medical Corporation on 400 subjects over a period of 3 months. The study was approved by the institutional review board and patients signed a consent prior to participation. The patients were grouped in 3 consecutive groups (group 1; $n = 107$; Group 2; $n = 197$; Group 3; $n = 96$) to evaluate the reproducibility of ORP.
Participants/materials, setting, methods: Exclusion criteria included azoospermia, presence of STD or chronic disease, use of prescription or OTC medications or antioxidants. Samples were collected and semen parameters assessed using the WHO guidelines (5th edition, 2010). Parameters included: volume, total number, concentration, total motility, progressive motility, and morphology. sORP was measured (mV) using the MiOXSYS system and normalized to concentration (mV/10^6 sperm/mL). For group comparisons, only those samples with a concentration >0.999 × 10^6 sperm/mL were included.
Main results and the role of chance: There was no statistically significant difference between the 3 data sets as regards patients demographics, semen parameters or hormonal level. The sORP results showed high degree of correlation between 3 datasets which means that a single test reading can provide reliable results.

Significantly higher sORP values were seen in semen samples with abnormal sperm parameters compared to normal in the three data sets with regards to total sperm count, sperm concentration, percentage total motility, progressive motility and percentage normal sperm morphology. Based on data set 1, an sORP cut off value of 3.29 mV/106 sperm/mL was determined to differentiate between semen samples with normal and abnormal sperm morphology percentage. This cut off value was confirmed as being reliable using data sets 2 and 3 with an overall specificity of 89.1% and PPV of 85.7%.
Limitations, reasons for caution: The current study was performed out of a single fertility clinic and since semen parameters as well as the redox system are subject to many lifestyle variables (ie. regional diets and lifestyles) it would be beneficial to explore the sORP values in regards to semen quality using culturally diverse populations.
Wider implications of the findings: sORP levels may be used as a new key perfomance indicator of sperm quality. sORP levels can be determined in patients with different diseases like Varicocele or infection and may help to identify the benefit of using antioxidant supplementation.
Trial registration number: Not a clinical trial.

P-014 Clinical value of ultrasonographic (US) analysis of seminiferous tubules for predicting successful sperm retrieval in patients with non-obstructive azoospermia (NOA)

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Study question: Can US analysis of seminiferous tubules predict the outcome of microdissection testicular sperm extraction (micro-TESE) in patients with NOA?
Summary answer: Thick seminiferous tubules (over 300 μm in diameter) on US images may help predict successful sperm retrieval on micro-TESE in patients with NOA.
What is known already: Micro-TESE has become a technique of choice for retrieving sperm in NOA. At present, however, there are no reliable predictors of the presence of testicular sperm in NOA patients. The concept that underlies micro-TESE is that seminiferous tubules containing germ cells are likely to be larger and more opaque than tubules without sperm production. To our knowledge, there has been no previous US analysis of seminiferous tubules with the aim of predicting successful micro-TESE.
Study design, size, duration: This prospective observational study involved 621 patients with NOA or cryptozoospermia who underwent micro-TESE in our private clinic from July 2003 to December 2015. Endocrinological measurements and US images of the testis were obtained before surgery, and compared with the results of micro-TESE.
Participants/materials, setting, methods: We evaluated 621 patients who underwent micro-TESE. The preoperative evaluation included serum FSH, LH, testosterone measurement and US evaluation of the testis, which involved measurement of the testicular volume and the diameter of seminiferous tubules. US was performed using a 10- or 14-MHz linear array transducer. To optimize US images for visualization of the seminiferous tubules, gain and contrast were appropriately adjusted with graphic software.
Main results and the role of chance: Seminiferous tubules over 200 μm in diameter can be visualized by our method, and we defined those of more than 300 μm in diameter as thick seminiferous tubules. Testicular sperm were retrieved successfully in 128 of the 215 patients (60%) with thick seminiferous tubules observed by US. On the contrary, testicular sperm were found by micro-TESE in only 35 of the 406 patients (9%) whose seminiferous tubules were under 300 μm in diameter. When we used a cut-off value for the seminal tubule diameter of over 300 μm, the sensitivity and specificity for predicting a successful outcome of micro-TESE were 0.79 and 0.81, respectively. Age, serum testosterone, FSH, LH and testicular volume were not correlated with the surgical detection of sperm by micro-TESE.
Limitations, reasons for caution: In patients with intrascrotal calcification including severe testicular microlithiasis, testicular parenchyma cannot be assessed by US.
Wider implications of the findings: US analysis of seminiferous tubules can provide valuable information for determining the extent of spermatogenesis in patients with hypogonadotropic hypogonadism being treated with gonadotropins.
Trial registration number: Not required.

P-015 Randomized correlations of trace elements with reproductive hormones and semen quality among Sudanese infertile males

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Study question: To correlate the serum and semen concentrations of trace elements with reproductive hormones and semen quality among infertile Sudanese males.
Summary answer: The seminal cadmium and lead concentrations had affected the spermogram and reproductive hormones levels, and their serum concentrations had correlated with these parameters.
What is known already: The concentrations of trace elements significantly correlate with azoospermia, oligospermia, and abnormal sperm motility among infertile males. The reproductive hormones levels in male infertility reflect a positive relationship with trace elements concentrations in seminal and serum specimens; and specifically strongly correlated between the luteinizing hormone and seminal cadmium in azoospermic patients.
Study design, size, duration: The study was a case finding hospital, laboratory-based, and prospective. It is a comparative, cross-sectional, quantitative study. 500 infertile males were enrolled in this study that was carried out in Khartoum (Sudan) within a period of two years. The patients studied were infertile males The patients were divided into four groups: 150 azoospermic, 150 oligospermic, 100 asthenozospermic patients, and 100 patients with abnormal sperm morphology. Control group included 100 fertile males.

Participants/materials, setting, methods: A structured interview was conducted to collect demographic and reproductive history. A complete physical and andrological examination was carried out. Semen samples were obtained by masturbation. Serum and semen samples were analyzed for reproductive hormones and trace elements. The reproductive hormones were assayed by ELISA technique; and trace elements were measured by atomic absorption spectrometry. Data were expressed in mean, standard error, standard deviation, and analyzed by the *t*-test, one- way analysis of variance.
Main results and the role of chance: Seminal cadmium concentrations were significantly increased in both azoospermic and oligospermic patients ($p < 0.05$). Seminal lead concentrations showed an insignificant elevation in azoospermic and oligospermic patients ($p < 0.05$). Magnesium and calcium were significantly reduced in azoospermic and oligospermic patients ($p < 0.05$). Manganese, cobalt, chromium and copper concentrations showed no significant variations in seminal plasma. In serum, cadmium concentration was significantly increased in azoospermic ($p < 0.05$), and other trace elements indicated variable concentrations fluctuating within normal range. Lead concentrations were well correlated in both serum and semen of azoospermic and oligospermic patients. Also semen and serum cadmium concentrations were significantly correlated in abnormal sperm motility patients ($p < 0.05$). Serum lead concentrations were well correlated with liquefaction times in azoospermic patients ($p < 0.05$). Significant correlation was recorded between luteinizing hormone and semen cadmium in azoospermic patients, between prolactin hormone and serum lead concentrations, and between follicle stimulating hormone and serum cadmium in abnormal sperm motility patients ($p < 0.05$).
Limitations, reasons for caution: No specific limitations or reasons for caution.
Wider implications of the findings: An increasing number of reports suggest that chemical and physical agents in the environment, introduced and spread by human activity, may affect male fertility.
Trial registration number: This research was approved by the Research Committee of Al Neelain University, Khartoum, Sudan. An oral consent was obtained from both infertile and control patients.

P-016 investigation of the effects of ion channel expression in infertile men patients

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Study question: To evaluate ion channel expressions in infertile man
Summary answer: These ion channels have an effect on sperm progressive motility and morphology in these ion channels may result in infertility.
What is known already: Infertility which is described as not receiving pregnancy despite unprotected and regular sexual intercourse in a one year period is detected by 15% of the couples. Male and female factor in the aetiology may be detected in similar rates. Especially idiopatic infertility which is recorded as 20% of the cases is a new topic of research.
Study design, size, duration: A hundred and twenty patients, 30 healthy controls were included the study between June 2014 and June 2015. they were divided into 5 equql groups. Group 1 was the normozoospermia (control) group, Group 2 consisted of oligozoospermic, group 3 asthenozoospermic, group 4 teratozoospermic and group 5 oligoasthenoteratozoospermic patients.
Participants/materials, setting, methods: We used total RNA Isolation, Spectrophotometric RNA Measurement, Complementary DNA Synthesis, DNA Amplification with Real Time Polymerase Chain Reaction, methods used for ıon channel assessment.
Main results and the role of chance: In our study we have detected a decrease in the gene expression of CatSper1, 2, 3, 4, KCNU1, Slo3, TMEM16A by asthenozoospermic patients and no significant difference in the Hv1 gene expression. The teratozoospermic group revealed a decrease in CatSper1, 2, 3, 4, KCNU1, TMEM16A and no significant difference in Hv1 and Slo3 gene expressions. By the oligozoospermia group an increase was observed in CatSper1, CatSper4, Hv1, Slo3, TMEM16A gene exspressions, no significant difference was detected in the gene expressions of CatSper2 and CatSper3 wheras a decrease was found in KCNU1 gene expression. The oligoasthenoteratozoospermia group revealed a decrease in CatSper1, CatSper4, Hv1, Slo3 and TMEM16A gen expressions, no difference in gene expressions of CatSper2 and KCNU1 and a decrease in CatSper3 gene expression.
Limitations, reasons for caution: There is a need for detailed evaluation in future studies with larger numbers to investigate this mentioned relation by oligozoospermic and oligoasthenoteraotozoospermic patients.
Wider implications of the findings: We believe that complete clarification of the ion channel functions and developing activator/inhibitör molecules targeting these channels could bring therapeutic options for patients with idiopathic infertility into existence. Also these activator/inhibitor molecules could affect solely on sperm cells and may be the precursors of drugs providing male contraception.
Trial registration number: Firat university local ethic commitee approved study with the registration number:30.10.2013/06-14.

P-017 The structure of human sperm transition proteins of normo- and subnormospermia and its relationship with DNA integrity and conventional sperm parameters

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Study question: What is the relationship between TNP1 and Protamine 1 and 2 in normo and subnormosperm and their relation to conventional sperm parameters?
Summary answer: There are a positive correlation between TNP1, protamine 2 and chromatin condensation and DNA fragmentation of spermatozoa.
What is known already: During spermiogenesis, haploid spermatids undergo complex morphological and physiological changes to differentiate into spermatozoa (Grzmil et al., 2008). These processes include chromatin remodelling mediated by the replacement of histones through protamines (PRM) and transition nuclear proteins (TNPs). Expression levels of PRM1 have been correlated with the DNA damage in mice (Suganuma et al., 2005), Inactivation of Tnps in mice generates a subfertile phenotype, showing less condensed sperm nuclei and elevated level of breaks in the DNA strand and other morphology defects (Yu et al., 2000).
Study design, size, duration: Prospective controlled trail.
Participants/materials, setting, methods: This study carried out at the department of Obstetrics and Gynecology University of Saarland.
20 noromospermia (G.1); and 30 with subnormospermia (G.2) men were enrolled in this study. Semen samples were prepared according to WHO guideline 2010 and sperm nuclear protein were extracted from each semen sample using acid-urea-PAGE and western blot as described previously by Mengual et al. (2003). Chromatin condensation was measured by Chromomycine A3 staining and DNA fragmentation evaluated by TUNEL assay.
Main results and the role of chance: The levels of TNP1 and protamines 1, 2 in normospermia (G.1) were (37.50 ± 3.64 ng/10^6; 416.2 ± 22.61 ng/10^6; 406.8 ± 21.53 ng/10^6) and the corresponding values for G.2 were (128.5 ± 11.89 ng/10^6; 429.7 ± 22.14 ng/10^6 and 323.8 ± 13.77 ng/10^6respectively). A significant differences between G.I and G.II were observed in the concentrations of TNP1 ($p = 0.0001$) and Protamine 2 ($p = 0.001$). Besides, TNP1 correlate significantly negative with sperm vitality ($r = -0.567$; $p = 0.0001$) and sperm motility ($r = -535$; $p = 0.001$) and correlate positively significant with chromatin CMA3 ($r = 0.390$; $p = 0.005$) and TUNEL value ($r = 0.340$; $p = 0.02$).
Beside, G.I showed a significantly higher ($p = 0.0001$) sperm count (78.00 ± 6.19 mill/mL) motility ($51.50 \pm 4.85\%$), vitality ($65.50 \pm 2.58\%$) and membrane integrity ($74.50 \pm 1.69\%$) in comparison to the values in G. II. (39.53 ± 5.34 mill/mL; $23.33 \pm 1.79\%$; vitality $36.33 \pm 2.57\%$; and $55.00 \pm 1.88\%$ respectively).
Limitations, reasons for caution: No limitations.
Wider implications of the findings: Funding from University of Saarland Budget.
Trial registration number: No registration.

P-018 A prospective double-blind randomized placebo-controlled study of the effect of vitamin E on semen parameters in infertile men

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Study question: To evaluate the efficacy of an oral vitamin E on semen quality parameters, administrated once daily for three months in infertile couples just prior to *in vitro* fertilization (IVF)cycles.
Summary answer: Our study has reported that treatment with vitamin E has a beneficial effect on sperm parameters and pregnancy rates after IVF cycles.
What is known already: Oxidative stress (OS) plays an important role in the pathophysiology of male infertility. Human spermatozoa are redox active cells capable of generating small amounts of reactive oxygen species (ROS) which are needed for maduration, capacitation, hyperactivación, acrosome reaction and oocyte fusion. ROS levels are continuously neutralized by antioxidant systems such as enzymes (superoxido dismutase (SOD), catalase, Glutatuhione System (GSH) and non-enzymatic antioxidants (vitamins (E, C, D), polyphenols (flavonoids) and trace mineral among others).

It has not been established yet the optimal dose of vitamin E, duration of treatment or the subpopulation of infertile patients who might benefit most from vitamin E therapy.
Study design, size, duration: This study was designed as a prospective, placebo-controlled and double blinded analysis of the *in vivo* effects of vitamin E, administered orally for 3 months in men undergoing *in vitro* fertilization cycles. It was perfomed at Cruces University Hospital in Spain from 2010 to 2014.

The study was approved by the Institutional Review Board of Cruces Hospital, and all participants signed written informed consent for the research.

The semen samples were obtained from 114 males subjects.
Participants/materials, setting, methods: 114 men were included in the study. Of these, 55 patients were treated with vitamin E (alpha-tocopherol) and 59 patients with placebo daily for three months.

The variables taken into consideration before and after antioxidant treatment were sperm concentration ($n \times 10^6$/mL), sperm count (10^6/ejaculate) and progressive motility (a + b%).

The patients were grouped into three categories such as normozoospermic, oligozoospermic and asthenozoospermic.

All patients were evaluated for sperm parameters according to the 2010 WHO criteria.
Main results and the role of chance: During the pretreatment phase no differences in baseline parameters or semen parameters were found between the placebo and vitamin E group.

After treatment, the sperm concentration ($n \times 10^6$/mL) had an improvement of 8.6% (68.9 ± 47.2 to 76.1 ± 40.1) in the placebo group and 23.8% (49.9 ± 41.7 to 72.3 ± 41.2) in the vitamin E group. The differences were statistically significant between groups ($p = 0.049$).

The percentage of progressively motile spermatozoa (a + b) increased a 5.8% (42.8 ± 19.5 to 49.1 ± 18.8) in the vitamin E group but in the placebo group (47.6 ± 17.4 to 46.2 ± 19.1) no changes were observed. The differences were statistically significant between groups ($p = 0.020$).

Although the sperm count (10^6/ejaculate) was improved in both groups (211.1 ± 188.5 to 221.2 ± 181.9 in placebo group and 164.4 ± 171.5 to 228.4 ± 187.0 in vitamin E group) the differences were not statistically significant ($p = 0.050$).

The pregnancy rate was 25.0% in placebo group and 45.2% in vitamin E group with differences statistically significant ($p = 0.049$).

When the patients of both groups (placebo and vitamin E groups) were divided into three categories as normozoospermic, oligozoospermic and asthenozoopermic some differences were found: 50% of oligozoospermic men improved sperm concentration ($n \times 10^6$/mL) ($p = 0.016$) and sperm count (10^6/ejaculate) ($p = 0.004$) at normozoospermic levels.

This trend was also observed in asthenozoospermic men but differences were not significant ($p = 0.057$).
Limitations, reasons for caution: Reduced sample size.
Wider implications of the findings: Understanding the physiologic and pathologic effects of free radical on sperm function will help in designing new and effective treatment strategies in male infertility. Our study has clinical relevance because the treatment with vitamin E shows a significant improvement in sperm motility and pregnancy rates comparing with placebo group.
Trial registration number: EudraCT 2007-000960-25.

P-019 Oxidation-reduction potential (ORP) of spermatozoa selected for intracytoplasmic sperm injection (ICSI) after exposure to polyvinylpyrrolidone (PVP) and hyaluronic acid (HA)

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Study question: Are there differences in the ORP of human spermatozoa exposed to PVP and HA prior to ICSI?
Summary answer: After exposure for 20 minutes and 1 hour, the lowest levels of ORP were noted in PVP-treated spermatozoa compared with the controls and HA-treated group.
What is known already: PVP is used in ICSI to facilitate sperm immobilization. It may, however, adversely impact the sperm function including DNA fragmentation, delays in calcium oscillations and loss of membrane integrity. Use of HA, a physiologic alternative to PVP, has been shown to help deselect sperm with DNA fragmentation, which may result in better embryo development after ICSI. ORP is a comprehensive measure of oxidative stress as it provides the overall balance between all available oxidants and antioxidants in a given sample. Static ORP (sORP) measures the current state of activity between oxidants and antioxidants.
Study design, size, duration: Human semen samples from healthy consented donors ($n = 11$) with normal semen parameters according to the 2010 WHO guidelines were used for sperm preparation by density gradient centrifugation. Washed specimens were incubated with either PVP (Origio, Denmark) or HA (SpermCatch, Nidacon, Sweden). Controls were treated with sperm wash medium only (Quinn's Advantage medium with HEPES; Origio, Denmark). sORP was measured after 20 minutes and 1 hour of exposure.
Participants/materials, setting, methods: Sperm preparation adjusted to 5×10^6/mL was added to PVP, HA or control group in a 3:10 ratio (vol./vol.). ORP was measured using a novel galvanostat-based technology with a MiOXSYS Analyzer (Aytu Bioscience, Englewood, CO) in triplicate at room temperature. Data were normalized and represented as mV/10^6 sperm for sORP. Paired-t test was used to compare the data and differences were considered statistically significant at the level of $p < 0.05$.
Main results and the role of chance: Sperm sORP levels differed significantly ($p < 0.05$) among the three groups after both 20 min and 1 h of exposure. The lowest sORP levels were noted in PVP-treated group followed by the HA-treated group and control group after 20 min and 1 h of exposure. Prior to any treatment with PVP or HA (Time 0), the sORP level in the control group (mean ± standard deviation) was 57.60 ± 0.63 mV/10^6 sperm. After 20 minutes, sORP levels were as follows: PVP-treated = 46.27 ± 0.37; HA-treated = 50.88 ± 0.15 and control = 57.49 ± 0.79, respectively. These values were not significantly different after 1 hour: PVP-treated = 47.04 ± 1.01; HA-treated = 50.80 ± 1.23 and control = 57.30 ± 2.66, respectively.
Limitations, reasons for caution: Samples were obtained from normozoospermic men. Further experiments involving sperm from infertile men may be helpful in confirming our findings. Embryo development and implantation potential of resulting embryos from sperm populations with different ORP were not assessed.
Wider implications of the findings: Levels of oxidative stress as represented by sORP levels were lowest in PVP-treated group, possibly because PVP contains a synthetic serum replacement – a chelating agent not present in HA. The findings indicate that PVP may have antioxidant properties and support continued use of PVP for sperm manipulation in ICSI processes.
Trial registration number: Not applicable.

P-020 Two-cell mouse embryo assay (MEA) outcome after intracytoplasmic injection of human sperm selected in polyvinylpyrrolidone (PVP) or hyaluronic adid (HA)

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Study question: Does immobilization in HA rather than the commonly used PVP affect the embryo development potential after intracytoplasmic sperm injection (ICSI) into mouse oocytes?

Summary answer: No significant differences were noted in oocyte degeneration rate and percentage of embryos at the two-cell stage among the PVP-treated, HA-treated and control groups.
What is known already: PVP is used to facilitate handling and immobilization of sperm before ICSI. However, it can adversely affect the sperm including loss of integrity of plasma membrane, acrosomal and mitochondrial membranes. Such damage induces deterioration of the chromatin, axonemal tubules, fibrous sheath, and accessory fibres. Also, this synthetic compound can cause sperm DNA damage, remain in the oocytes for a prolonged period after injection and further cause decondensation within the oocyte. HA, on the other hand, is a substance that naturally occurs in human reproductive tract, and some preliminary data suggest that it may represent a good physiologic alternative to PVP.
Study design, size, duration: Human semen samples with normal semen parameters according to WHO 2010 guidelines were prepared by density gradient centrifugation followed by sperm selection in PVP (Origio, Denmark) or HA (SpermCatch, Nidacon, Sweden) or culture medium only (Quinn's Advantage medium with HEPES, Origio, Denmark; control). Immobilized sperm from each group were microinjected into mouse oocytes to assess oocyte degeneration rate, and the percentage of embryos at the two-cell stage as surrogate for sperm decondensation.
Participants/materials, setting, methods: Frozen mouse oocytes ($n = 94$) were thawed and surviving oocytes ($n = 83$) were randomized into three groups for ICSI: PVP-treated ($n = 28$); HA-treated ($n = 27$) and control ($n = 28$). After ICSI, oocyte degeneration rate was noted and percentage of embryos at the 2-cell stage was assessed as surrogate for sperm decondensation. Chi-square test and Fisher's exact test were used to compare the data; $P < 0.05$ was considered statistically significant.
Main results and the role of chance: Of 94 mouse oocytes thawed, survival rate was 88% (83/94). The degeneration rates of oocytes measured 18 hours post ICSI were comparable across the groups ($P = 0.93$; Chi-square test): 32% (9/28) in PVP group, 33% (9/27) in HA group, and 29% (8/28) in control group. The survival rate of embryos at the two-cell stage 24 hours after ICSI was comparable across the groups ($P = 1.0$; Fisher's exact test): 74% (14/19) in PVP-group, 72% (13/18) in HA-group and 75% (15/20) in control group.
Limitations, reasons for caution: Further experiments involving a larger sample size may be helpful in confirming the findings of our study. Other outcome measures previously linked to the detrimental effect of PVP were not tested.
Wider implications of the findings: Our results demonstrate no significant differences in mouse oocyte degeneration rate and, the percentage of embryos at the two-cell stage assessed as surrogate of sperm decondensation among the PVP-and the HA-bound human sperm after ICSI. The use of HA may be a physiologic replacement for the synthetic compound PVP.
Trial registration number: Not applicable.

P-021 Combination of density gradient centrifugation and magnetic-activated cell sorting significantly reduce sperm DNA fragmentation index of semen samples

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Study question: Is it possible to reduce sperm DNA fragmentation index by sequential treatment of density gradient centrifugation (DGC) and magnetic-activated cell sorting (MACS)?
Summary answer: Combination treatment of DGC and MACS showed a significant synergy effect on reducing the DFI of semen samples.
What is known already: Density gradient centrifugation is widely used to collect the sperm with progressive motility from the raw semen. Recently, an inverse correlation between sperm DNA fragmentation and progressive motility was observed in our previous studies. MACS recently has been introduced for isolation of non-apoptotic sperm because apoptosis is well known to be a major cause of sperm DNA fragmentation. Although a few of authors reported a positive effects of DGC and MACS on reducing the sperm DFI, further studies need to investigate the effect of combination of DGC and MACS on isolation of the sperm with high DNA integrity.
Study design, size, duration: This is a prospective study performed from Jan. 2015 to Aug. 2015.
Participants/materials, setting, methods: 29 normozoospermic semen samples were obtained from the volunteers. Sperm with progressive motility were isolated from raw semen by DGC, and then the motile sperm was separated into apoptotic and non-apoptotic sperm by using MACS ART Annexin V system kit. Sperm DFI was evaluated with Halosperm test.
Main results and the role of chance: Sperm DNA fragmentation index (11.6%) of non-apoptotic sperm was significantly lower than that (21.1%, $P < 0.01$) of apoptotic sperm which means a positive effect of MACS on reducing the sperm DFI. An inverse correlation between sperm DFI and progressive motility was observed in both apoptotic ($r = -0.506$, $P < 0.01$) and non-apoptotic sperm ($r = -0.508$, $P < 0.01$). Density gradient centrifugation also significantly reduced the sperm DFI (11.5%) of raw semen down-to 8.1% ($P < 0.05$). The sperm DFI (11.5%) after DGC alone was significantly re-decreased to 4% ($P < 0.05$) after combination treatment of DGC and MACS.
Limitations, reasons for caution: The limitation in the number of the semen samples involved in this study which slightly reduced the statistical analysis power.
Wider implications of the findings: This combination treatment will be a practical and efficient method to isolate the sperm with high DNA integrity for the patients who showing high sperm DFI.
Trial registration number: None.

P-022 Addition of myoinositol into sperm preparation medium improved the sperm motility and pregnancy rate of IUI cycles in asthenozoospermic patients

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Study question: This study was performed to evaluate the effects of myoinositol (MI) on sperm motility and clinical outcomes of IUI cycles.
Summary answer: Myoinositol improved the sperm motility and pregnancy rate of IUI cycles in asthenozoospermic patients, but it didn't show any beneficial effect in normozoospermic patients.
What is known already: Percentage of spermatozoa with high mitochondrial membrane potential (MMP) levels showed a correlation with high fertilization rate because the MMP level of sperm is directly associated with motility of sperm. However the MMP level is inversely correlated with ROS level that induce a damage to the mitochondrial membrane. MI increased the percentage of spermatozoa with high MMP level in the patients with abnormal sperm parameters. Moreover, MI increased fertilization and pregnancy rates in women who failed to pregnancy due to poor oocyte quality by reducing oxidative stress in culture conditions.
Study design, size, duration: A prospective study was carried out to investigate the effects of MI on sperm motility and clinical outcomes in IUI cycles from August to December 2015. Total 376 patients (IUI cycles) were divided into two groups according to the addition of MI into sperm preparation medium; MI group ($n = 144$) and Control group ($n = 232$). Both groups were subdivided into 3 groups according to the result of semen analysis; Normozoo-, Asthenozoo-, and Oligoasthenozoo-spermic patient groups.
Participants/materials, setting, methods: Raw semen was treated with density gradient centrifugation for isolation of motile sperm, and then the motile sperm was washed twice with Ham's F-10 with or without 5mg/ml MI. An antioxidant effect of MI on the sperm motility suppressed by hydrogen peroxide was investigated to identify the enhancing effect of MI on motility.
Main results and the role of chance: The effects of various MI concentrations (0, 2, 5, 10, 20 mg/ml) on motility of raw semen was investigated. Even the treatment with low concentration of 5mg/ml significantly increased the motility (38.1%) compared to the treatment without MI (21.5%, $P < 0.01$). Unlike the raw semen, however, in the motile sperm isolated by density gradient centrifugation, the sperm motility in the MI group was not different from the motility in the control group (87.1% vs 83.5%). Between the MI and Control groups, there were no differences in the age of patients (female; 33.7 vs. 34.1, male; 35.9 vs.35.9), volume (3.4 vs. 3.3ml), count (89.5 vs. 101.8×10^6/ml), and motility (42.1 vs. 47.3%) of the raw semen. The pregnancy rates of asthenozoo- (28.8%) and oligoasthenozoo-spermic patients (33.3%) in the MI group were higher than those of control group (18.8 and 25.0%, respectively), although a statistical significance was not observed in the difference. Addition of hydrogen peroxide

significantly decreased the sperm motility in a concentration-dependent manner. The suppressive effect of hydrogen peroxide was significantly reversed by addition of MI.
Limitations, reasons for caution: The limitations of the present study included the limited number of samples tested, which slightly reduced the study's statistical power.
Wider implications of the findings: MI improved sperm motility and pregnancy rate of IUI cycles in asthenozoospermic patients. The beneficial effect of MI may be associated with antioxidant activity as well as improvement of MMP level. Therefore, MI also may be useful for IVF-ET cycles in asthenozoospermic patients.
Trial registration number: None.

P-023 PAWP expression and distribution in spermatozoa is not associated with fertilization failure

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Study question: Is PAWP protein expression and distribution in sperm cells associated with fertilization failure in cycles with donated oocytes?
Summary answer: PAWP protein expression and distribution in sperm cells is not associated with fertilization failure in cycles with donated oocytes.
What is known already: Successful fertilization in mammals depends on sperm cell's ability to initiate intracellular Ca^{2+} oscillations in the oocyte. This phenomenon is elicited by a Sperm Oocyte Activating Factor (SOAF), whose quantitative and/or qualitative defect should result in fertilization failure. Post-Acrosomal WW domain-binding Protein (PAWP) has been proposed as a putative SOAF, but its ability to activate oocyte has been questioned in the recent literature in a rodent model of fertilization, and its implication in fertilization failure remains unknown. Identifying and studying SOAFs in the human species is particularly difficult as oocyte factors contributing to the phenotype cannot be usually excluded.
Study design, size, duration: This monocentric prospective cohort study was conducted in 2015 on 8 couples referred either for an autologous cycle with woman's own oocytes (4 couples) or for a cycle with either donor oocytes following female ovarian insufficiency (4 couples). In all cases, elective ICSI was performed, resulting in fertilization failure (<10% fertilization rate). All oocyte donors were of proven fertility. A cohort of 8 sperm donors with proven fertility was used as control.
Participants/materials, setting, methods: In each male patient and donor, PAWP protein expression was analyzed by western blot (WB; relative expression versus α-tubulin) and immunofluorescence (IF; proportion of spermatozoa with a PAWP signal in the equatorial region) with Abcam ab170115 antibody on raw frozen-thawed sperm sample used for ICSI. PAWP expression and distribution in patients and donors were compared with Mann Whitney rank test.
Main results and the role of chance: Mean fertilization rate was 5% (range [0–10%]) in patients and 68% in donors, (range [33–88%]). PAWP was present in all patients' sperm samples and in all sperm donors. Mean PAWP expression in WB was not significantly different between patients with fertilization failure (mean 1.12 ± 0.35; range [0.82–1.89]) and fertile semen donors controls (mean 1.25 ± 0.45; range [0.74–1.81]). The proportion of PAWP positive sperm cells in IF was not significantly different between patients and donors (51.2% ± 27.9 vs 65.7% ± 12.9 respectively). This result remained the same when only sperm cells with visible acrosome instead of all sperm cells where considered for PAWP staining (55.5% ± 25.2 vs 66.2% ± 12.6 in patients and donors respectively).
Limitations, reasons for caution: Although we aimed to isolate fertilization failure cases of male origin by integrating cycles with oocytes donated from young fertile donors, and elective ICSI to ensure sperm entry, we cannot exclude completely an unknown oocyte effect on the observed fertilization outcomes.
Wider implications of the findings: The results of our study do not support the hypothesis of a clear role of PAWP as SOAF in the human species. Likewise, the study of PAWP protein expression in sperm cells as a diagnostic or prognostic marker of fertilization outcomes is not supported.
Trial registration number: not applicable.

P-024 PAWP gene and protein expression in sperm cells in relation to sperm parameters and ICSI outcome in egg donation cycles

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Study question: Is PAWP protein and gene expression related to sperm parameters and oocyte donation cycle outcome?
Summary answer: PAWP gene and protein expression were neither correlated with semen analysis parameters, nor with oocyte donation cycle outcome.
What is known already: Post-Acrosomal WW domain-binding Protein (PAWP), a protein found in the equatorial region of mammalian spermatozoa, has been proposed as a Sperm borne Oocyte Activating Factor contributing to calcium release within the oocyte and subsequent fertilization and embryo development. However, its capability to elicit calcium oscillations individually in oocytes and its relevance as either a diagnostic or a prognostic marker of fertilization failures has been questioned in the recent literature.
Study design, size, duration: This basic research study was conducted in 2015 in 33 couples referred for oocyte donation cycle. PAWP protein and gene (when possible) expression was measured in each patient on raw frozen-thawed semen sample.
Participants/materials, setting, methods: Western blot (WB; relative expression versus α-tubulin) and immunofluorescence (IF; proportion of PAWP positive cells in equatorial region) were performed with Abcam ab170115 antibody on raw frozen-thawed sperm sample. In addition, PAWP gene expression was also measured in the same sperm samples by RT-qPCR for 20 patients presenting with cells for additional RNA extraction. PAWP gene and protein expression were then correlated with semen analysis parameters (number, motility and morphology) and oocyte donation cycle outcome.
Main results and the role of chance: PAWP protein was expressed in all patients with a high variability (mean relative expression 1.77 ± 0.8, range [0.4–3.7], mean proportion of positive cells 63.6% ± 13.9, range [37–85]). The analysis of PAWP expression with IF gave similar results in all sperm cells and in acrosome-visible (intact or reacted) cells (63.6% ± 13.9 vs 67.6% ± 12.1 respectively), confirming that PAWP localization and expression are equatorial, not acrosomal. PAWP gene was also expressed in all tested patients with a high variability (mean gene expression after normalization with geNorm algorithm 7.3 ± 8.2, range [0.2–13.7]). Gene expression levels were not significantly correlated with PAWP protein expression (R^2<0.1). Mean PAWP protein expression was comparable between normozoospermic patients and patients with abnormal sperm analysis. No significant correlation was found between PAWP gene or protein expression and sperm parameters (R^2<0.1 for every parameter) nor with fertilization rate. PAWP gene and protein expression was not significantly different according to oocyte donation clinical outcome, i.e. pregnancy achievement or not.
Limitations, reasons for caution: Analysis of PAWP expression with WB was semi-quantitative.
Wider implications of the findings: This study does not support the interest of PAWP protein and gene expression in sperm cells as a prognostic factor of ICSI cycle outcome.
Trial registration number: NA.

P-025 Effect of microsurgical varicocelectomy on semen parameters in severe male factor infertility

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Study question: This study was conducted to assess the effect of surgical repair of varicoceles on spermatogenesis, particularly in patients with severely compromised semen parameters.
Summary answer: Microscopic varicocelectomy provided a beneficial effect on sperm concentration and/or sperm motility consequently improving total motile sperm count as well as on sperm DNA integrity.
What is known already: Varicocele is one of the main reasons for male infertility, contributing to impaired spermatogenesis and decreased serum testosterone level. Also, it has been reported that varicocele induces sperm DNA damage. Varicocelectomy has been an effective therapeutic option in male infertility.

Study design, size, duration: Retrospective study. The subjects were separated into three groups of microsurgical varicocelectomy cases, from Nov. 2012 to Mar. 2015. Group 1: 24 cases with sperm concentration (SC) less than 5 million/ml; Group 2: 20 cases with sperm motility (SM) less than 20%, Group 3: 10 cases with sperm DNA fragmentation rate being higher than 50%.
Participants/materials, setting, methods: We compared semen parameters before microsurgical varicocelectomy and 3 months following the surgery among three groups. Sperm DNA integrity was assessed by sperm chromatin dispersion test (Halosperm®). Clinical outcome was evaluated in cases where post-surgery progress follow-up was possible.
Main results and the role of chance: In Group 1, the SC, SM and total motile sperm count (TMSC) before surgery were $2.0 \pm 1.7 \times 10^6$/ml, 31.5 ± 21.7%, and $5.4 \pm 21.7 \times 10^6$, respectively, while post surgery, they were $9.8 \pm 12.3 \times 10^6$/ml, 40.2 ± 25.2%, and $16.9 \pm 21.5 \times 10^6$. The post SC and TMSC were significantly improved ($P < 0.05$). In Group 2, the SC, SM and TMSC prior to surgery were $15.4 \pm 15.0 \times 10^6$/ml, 10.0 ± 6.7%, and $4.8 \pm 4.4 \times 10^6$, respectively. While post surgery, they were $23.8 \pm 19.3 \times 10^6$/ml, 30.5 ± 26.0%, and $29.0 \pm 37.9 \times 10^6$, respectively. The post SM and TMSC were remarkably increased ($P < 0.05$). As for Group 3, although the post SC, SM and TMSC were similar to those before the treatment, the post DNA fragmentation rate (42.1 ± 18.8%) was significantly reduced ($P < 0.01$) from that of before surgery (73.7 ± 14.7%). Among those patients, 8 females became pregnant during a follow-up period.
Limitations, reasons for caution: None.
Wider implications of the findings: Surgical repair of varicoceles contributed to improvement in the TMSC and the sperm DNA integrity. Furthermore, pregnancies were obtained with improved semen, suggesting that microsurgical varicocelectomy is an effective strategy for males with severely compromised spermatogenesis.
Trial registration number: None

P-026 Proliferation of spermatogonium GC-1 spg and GC-2 spd cells was regulated by SET protein

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Study question: Whether SET protein participates in the regulation of spermatogenesis
Summary answer: SETprotein participates in the regulation of spermatogenesis
What is known already: SET protein is mainly expressed in spermatogonial cells and spermatocytes, suggesting that it is involved in spermatogenesis. In this study, we find SET protein inhibite proliferation and cell cycle of GC-1 spg and GC-2 spd cells. SET selectively regulates Akt phosphorylation at Ser-473 to regulate cell proliferation.
Study design, size, duration: The cultured GC-1 spg and GC-2 spd cells were transfected with AdH1 -siRNA/SET. Proliferation, cell cycle, AKT, pAKT (S473) and pAKT (T308) were investigated.
Participants/materials, setting, methods: The expression and cellular location of SET protein was assessed by Immunofluorescence and Confocal laser scanning microscopy. Western Blotting was used to detect the expression level of SET protein before and after transfection. Cell viability was determined by CCK8 assay. Cell proliferation was tested by BrdU incorporation assay, while cell cycle were measured by flow cytometry. Expressions of AKT, pAKT (S473), pAKT (T308)were determined by Western Blotting.
Main results and the role of chance: In GC-1 spg and GC-2 spd cells, SET protein was found in both the cytoplasm and nucleus, but its location was mainly in the cytoplasm. Expression of SET Protein in the GC-1 spg and GC-2 spd cells transfected with AdH1 -siRNA/SET was significantly lowered, while the cell viability and the proliferation rate was decreased (all $P < 0.01$). Knockdown of SET protein, the percentage of S phase was decreased in both GC-1 spg and GC-2 spd cells. An increase of percentage of G2/M phase in GC-1 spg and percentage of G0/G1 and G2/M phase in GC-2 spd cells were observed ($P < 0.05$). Down-regulated SET expression for 48h, the expressions of pAKT (S473) was decreased but not pAKT (T308) ($P < 0.05$).
Limitations, reasons for caution: More animal experiments are needed to support our study.
Wider implications of the findings: SET may be a potential therapeutic target in oligo-astheno-teratospermia for future clinical application.
Trial registration number: NO.

P-027 Simple effective vitrification of a small number of human spermatozoa using Rapid-i carriers: a follow-up study

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Study question: To determine the efficacy of our technique for vitrifying a small number of sperm, from 35 severe oligosperimic/azoospermic patients, followed by ICSI with warmed sperm.
Summary answer: The procedure yielded high post-warm sperm recovery rates, normal fertilization, and good quality (GQ) embryos, resulting in six successful pregnancies after ET of these embryos.
What is known already: Despite interest in low sperm count cryopreservation for men with severe male factor infertility, little is available regarding the ideal carrier or technique that can be used universally. Several commercial devices have been applied for cryopreserving such sperm in miniscule fluid volumes to facilitate recovery, but the sample size was small, and reports of subsequent successful delivery are limited to case reports (Desai et al., 2012; Endo et al., 2012). We previously described a novel, simple method for vitrifying a limited number of human sperm using the closed system Rapid-i (ESHRE 2011) and subsequently implemented the technique at our clinic.
Study design, size, duration: Vitrification of a small number of sperm was performed for 28 men with severe oligospermia/azoospermia following testicular biopsy (Group A) and for 7 men with severe oligospermic semen samples (Group B) from November 2011–December 2015. ICSI treatment was performed with the vitrified/warmed sperm (52 cycles). The resultant 2PN oocytes/GQ embryos were vitrified by day 6. Data on ET of the warmed embryos performed by the end of December 2015 were analysed.
Participants/materials, setting, methods: Vitrification: 1–14 spermatozoa were aspirated into an ICSI pipette and deposited into 1.5 µL cryoprotective solution (2:1 mixture K-SISC:K-SISM buffers; Cook) on a Rapid-i carrier strip (Vitrolife), which was placed in LN_2 vapour (2 min), inserted into a pre-cooled RapidStraw, ultrasonically sealed, and plunged into LN_2. Warming: the cryopreserved strip was placed in 2 µL cryoprotective solution. Spermatozoa were recovered using an ICSI pipette and placed in 2 µL modified HTF medium for ICSI treatment.
Main results and the role of chance: In Groups A and B, 770 sperm (motile, 86.3%) and 155 sperm (motile, 100%) were vitrified in 140 and 36 Rapid-i carriers, respectively (5–81 sperm/man). The vitrified/warmed sperm of Groups A and B underwent 42 and 10 cycles, respectively, of ISCI. During ICSI, 485 sperm (85 carriers) and 100 sperm (23 carriers) were warmed, and the recovery rates were 87.6% and 97.0% in Groups A and B, respectively ($P < 0.01$). Motile sperm rates/recovered sperm were 53.9% and 41.2% in Groups A and B, respectively. Fertilization rates/ICSI with the recovered sperm were 99/247 (40.1%) and 22/44 (50.0%) in Groups A and B, respectively. The numbers of vitrified 2PN oocytes, GQ day 2/3 embryos, and GQ day 5/6 blastocysts were 35, 21, and 1 in Group A and 5, 3, and 1 in Group B. Groups A and B underwent 21 and 4 vitrified ET cycles, respectively. All warmed 2PN oocytes and embryos survived. Mean numbers of embryos transferred, clinical pregnancy rates/vitrified ET, and miscarriage rates/pregnancy were 1.38 ± 0.11, 8/21, and 3/8, respectively, in Group A, and 1.5 ± 0.29, 1/4, and 0/1 in Group B. Five and one singleton babies were live-born in Groups A and B, respectively.
Limitations, reasons for caution: Our Rapid-i method is far superior to the conventional (e.g., vial) methods for single sperm cryopreservation, as it helps circumvent a laborious and time-consuming search for individual sperm. However, long-term follow-up studies for more patients with very low sperm counts in their semen/testicular specimens are warranted to validate the efficacy.
Wider implications of the findings: Our new vitrification system should be valuable for severe male factor infertility. For example, effective sperm storage by this method prior to IVF cycles can avoid the complications and expense associated with repeated surgical sperm retrievals and the risk of finding no sperm on the day of oocyte retrieval.
Trial registration number: N/A.

P-028 Connexin 43 regenerates meiosis and toxicant-induced blood-testis barrier disruption in the rat testes

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Study question: We sought to examine if the gap junction protein connexin 43 (Cx43) would reseal disrupted blood–testis barrier (BTB) and reinitiate spermatogenesis in adjudin-treated rats.
Summary answer: It was found that overexpression of Cx43 indeed reboots meiosis and reseals BTB disruption, even though it fails to support round spermatids to enter spermiogenesis.
What is known already: We have shown that treatment of adult rats with an acute high-dose of adjudin [1-(2,4-dichlorobenzyl)-1H-indazole-3-carbohydrazide], a male contraceptive under investigation, induces irreversible BTB disruption. In these animals, the population of undifferentiated spermatogonia and spermatogonial stem cells remains largely unaffected, yet these spermatogonia fail to differentiate into spermatocytes. Interestingly, it has been found that Sertoli cell-specific deletion of the gap junction (GJ) protein Cx43 in mice leads to spermatogenesis failure and BTB in these Sertoli cell-specific Cx43 knockout mice also displays significant defects. Collectively, these findings illustrate the likely involvement of GJ in BTB function and its significant role in spermatogenesis.
Study design, size, duration: We expand our earlier study using the acute adjudin animal model to investigate if an overexpression of Cx43 in these rats would rescue spermatogenesis in particular meiosis and if the overexpression of Cx43 in these rats would reseal the disrupted BTB. If it did, we also sought to understand the underlying molecular mechanism (s) by which Cx43 regulate BTB function.
Participants/materials, setting, methods: A single acute dose of adjudin at 125 mg/kg body weight by oral gavage caused sterility in adult rats in which >98% of the tubules were devoid of germ cells except for the population of spermatogonia, which remained relatively unaffected. A full-length Cx43 cloned into mammalian expression vector pCI-neo was used to transfect testes of adjudin-treated rats versus empty vector.
Main results and the role of chance: Overexpression of Cx43 in the testis *in vivo* at 12, 12.5, and 13 wk in rats ($n = 4$ at each time point) vs. rats transfected with empty pCI-neo vector was found to induce resumption of meiosis. Most importantly, it was noted that round spermatids were detected in a significant number of tubules in testes overexpressed with Cx43 vs. adjudin-treated rats overexpressed with empty vector alone or rats treated with adjudin alone. However, elongating and elongated spermatids remained undetected in Cx43-overexpressed testes, illustrating spermiogenesis remained arrested. Immunofluorescent staining indicated that Cx43 in the overexpressed testes was localized prominently at the BTB and became properly organized. It was also noted that the resumption of meiosis was accompanied by reorganization of F-actin, anchoring junction proteins (N-cadherin and γ-catenin), and tight junction proteins (occludin and ZO-1) at the BTB. These studies were demonstrated by immunofluorescent and immunoblot analyses. By the use of a BTB integrity functional *in vivo* assay, we next examined whether the disrupted BTB induced by adjudin was resealed following overexpression of Cx43 in the testis. It was noted that the number of tubules in which the BTB displayed signs of being resealed was >80% in term with the transfection efficiency.
Limitations, reasons for caution: In summary, we have demonstrated that overexpression of Cx43 in the testis can reseal the adjudin-induced BTB disruption by conferring proper localization of anchoring and tight junction proteins to the BTB microenvironment. This also repairs spermatogenesis, at least through meiosis.
Wider implications of the findings: These findings suggest that toxicant-induced BTB dysfunction and male infertility including humans induced by other toxicants, such as cadmium, phthalates, and perfluorooctanesulfonate (PFOS), can possibly be therapeutically managed by overexpressing Cx43 because these toxicants were shown to exert similar effects both in rodents and humans in our recent studies.
Trial registration number: N.A.

P-029 Investigation the effects of chronic mobile phone radiation and melatonin used for protection purpose on the semen morphology

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Study question: What is the possible protective effects of melatonin on the semen morphology in the case of exposure to chronic mobile phone radiation?
Summary answer: Melatonin could be used potentially for inhibiting the mobile phone radiation, but it is not sufficient to prevent all of the spermium abnormalities.
What is known already: The mobile phone radiation causes some effects on the number, motility, viability and structure of spermia, and these criterias are very important for male fertility.
Study design, size, duration: 24 male rats were divided into 4 equal groups. There was no application performed to control group. The second group was injected by 10 mg/kg melatonin subcutaneously. The third group was exposed to 2100 MHz radiation for 30 minutes. The fourth group was injected by melatonin subcutaneously 40 minutes before the exposure to radiation for 30 minutes. All the groups were treated for 90 days respectively.
Participants/materials, setting, methods: 24 male Wistar albino rats were used for this study. By the end of the experiment, left caudal epididymis and ductus deferens tissues were taken and moved into Pure Sperm Wash (Nidacon) solution and dissected. Spermiogram, Spermac stain and Transmission Electron Microscobic examinations and statistical analyses were used. Perinuclear ring of the manchette and the posterior portion of the nucleus measurement (ARC) was performed for the heads of the spermia for all groups.
Main results and the role of chance: The light microscobic evaluations showed that the number of total spermiums, total motile spermia and active motile spermia were decreased in the other groups when compared to the control group. However, this reduction was statistically significant only in the radiation and radiation + melatonin treated groups. The number of spermia with normal morphology was also decreased significantly in the radiation and the radiation + melatonin treated groups. ARC measurements of the radiation and the radiation + melatonin treated groups showed a significant angular increase compared to the control group ($p < 0.05$). Evaluations of fhe findings obtained from the electron microscopy showed that the structures in the heads, the necks and the tails of the spermia in the melatonin treated group protected their normal pattern as the control group. The deformed regions in the acrosomes, derangement in some parts of the membranes, fussion of the nucleus content of acrosomes and degenerations in outer dense fibers were seen in the radiation treated group. The radiation + melatonin treated group had the acrosome and the nucleus with normal morphology; however, there were some separations in the membrane.
Limitations, reasons for caution: Melatonin injections have given a burning sensation to the animals so researchers must be careful for the reaction of subjects during the administrations. And also researchers must be protect themselves during the radiation applications.
Wider implications of the findings: No morphological and ultrastructural studies have been done for protective effects of melatonin in case of exposure to chronic mobile phone radiation on semen until this study and we found that melatonin can be used for inhibiting the radiation, but it is not sufficient to prevent all of the abnormalities.
Trial registration number: None.

P-030 Determination of an inducer optimized to induction of sperm capacitation in the mice

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Study question: What is type and concentration of an inducer showing the best capacitation rate among diverse sperm capacication inducers in the mouse sperms?
Summary answer: 2.7mM calcium was the most effective capacitation inducer among diverse sperm capacitation inducers,
What is known already: Acquisition of sperm capacitation in the female reproductive tract after ejaculation is an essential step in the fertilization with oocytes. Accordingly, during *in-vitro* fertilization, induction of capacitation in the retrieved sperms should be required necessarily for successful fertilization. To date, many candidate substances have been considered as capacitation inducers

in-vitro. However, there were no noticeable reports about the comparison of efficiency inducing sperm capacitation among diverse capacitation inducers.
Study design, size, duration: We tried to determine an inducer showing the best capacitation rate in the mouse sperms through comparative analysis.
Participants/materials, setting, methods: Different concentration of bovine serum albumin (BSA; 0.15, 0.30 and 0.45% (w/v)), calcium (0.9, 1.8 and 2.7 mM), progesterone (7.5, 15 and 30 μM), heparin (25, 50 and 75 μmol/ml), lysophosphatidylcholine (Lyso-PC; 50, 100 and 200 μM) as candidate capacitation inducers were adjusted to sperms collected in epididymis, and then ratio of sperms experiencing acrosome reaction observed in sperms acquiring capacitation was accessed using coomassie G-250 blue staining.
Main results and the role of chance: Although there were no significant differences among different concentration groups, 0.3% (w/v) BSA, 15 μM progesterone, 50 μmol/ml heparin and 50 μM Lyso-PC showed numerically the best induction of acrosome reaction in sperms. Moreover, sperms experiencing capacitation by 2.7 mM calcium showed significantly the highest percentage of acrosome reaction, compared to those by 0.9 and 1.8 mM calcium. Subsequently, in comparison of the ratio of sperms acquiring capacitation by each inducer showing the best potential in the induction of acrosome reaction, significantly the highest percentage of sperms with acrosome reaction was detected in the use of 2.7 mM calcium inducer.
Limitations, reasons for caution: The results of this study were derived from only mouse. Therefore, studies on determining type and concentration of an inducer showing the best capacitation rate among diverse sperm capacication inducers in the human and other species sperms should be conducted in the future.
Wider implications of the findings: This will greatly contribute to improvement of IVF success rate in the level of experimental animals.
Trial registration number: –

P-031 Pollution exposure and high DNA fragmentation index in human sperms: a case-control study

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Study question: Could pollution exposure play a key role on sperm quality, in terms of DNA fragmentation index (DFI)?
Summary answer: Patients exposed to high level of pollution show an higher percentage of sperm DNA fragmentation index in contrast with patient from control group.
What is known already: Taranto area is characterized by a number of steel factories and petrochemical industries. Data about the detrimental effects of environmental pollution are alarming. Pollution coming from the industrial plants causes health and fertility risks, mainly due to the exposure to several pollutant (PM10, heavy metals, etc).

Toxic substances can affect DNA directly or indirectly, through oxidative stress, impairing sperm quality: high levels of sperm DNA fragmentation index has been summoned as possible cause of male infertility; it is known, in effect, that a spermatic DFI less than 15% is physiologic, while above 30% is related to fertility issues.
Study design, size, duration: Case-control study: valuation of DFI in patients exposed to different level of environmental pollutants.

A. Patients from the city of Taranto leaving far from industries
B. Patients working in local steel factories in Taranto
C. Patients from Palermo (control group)

Duration of the study: 24 months, from January 2010 to December 2012.

DFI assessed by *in situ* TUNEL assay. The fields of fixed semen samples were analysed, by fluorescence and light microscopy.
Participants/materials, setting, methods: Three different groups of patients (tot. number of pts 152), afferent to an assisted reproduction clinic in Taranto: Group A) workers of local steel factories (pt. No: 28) and B) Taranto residents (pt. No: 61) and a Control group C) (pt. No: 63) afferent to an assisted reproduction clinic in Palermo with supposed fertility issues.
Main results and the role of chance: Our study analyzed sperm samples from three patients groups: A) workers of local steel factories; B) Taranto residents; C) Controls.

We observed a highly statistically significant increase of spermatic DNA fragmentation Index (DFI) in the "factory workers" group, constantly exposed to environmental pollutants for professional reasons" compared with control (DFI 31% *vs* 16.8% $P \leq 2.685 * 10^{-6}$) as well as in comparison with the Taranto resident (DFI 31% *vs* 25% $P \leq 2.9 * 10^{-3}$). We have also observed a statistically significant difference between Taranto resident and control group (DFI 25% vs 16.8% $P \leq 3.681*10^{-6}$).
Limitations, reasons for caution: The limitation of this study is that it is a retrospective study and the size of cohort of patients.
Wider implications of the findings: Our study supports the hypothesis that the level of dioxin exposition might be positively correlated with the sperm DFI.

Interrupting the sperms damaging source might bring back the DFI level to normal values. So, moving away from the damaging source, patients from A and B groups could restore spermatogenesis.
Trial registration number: The trial is an observational study and no registration is needed.

P-032 Relationship between leucospermia and sperm DNA damage

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Study question: Is there any relationship between leucospermia and sperm DNA damage?
Summary answer: There is a powerfull correlation between leucospermia and DNA damage.
What is known already: 15% of couples who are trying to achive a clinical pregnancy and had no pregnancy at least 12 months had an infertiliy problem. 50% of infertility is related to male factor. Some of these problems are curable and protectable that makes the diagnosis and treatment of male infertility is more valuable. One of the male factor of infertility is urogenital infections. Urogenital infections can effect sperm quality and so pregnancy.
Study design, size, duration: This prospective study was conducted in Andrology clinic of VM Medical Park Kocaeli Hospital during July to December 2015. A total of 10 men who failed to have a child despite 1 year of attempt were included in the study. 5 of them had leucospermia and the other 5 had no leucospermia. The sperm analysis were performed based on the World Health Organization (WHO) 2010 criteria.
Participants/materials, setting, methods: In this study only who had leucospermia and who had normal sperm parameters were included. Peroxidase test was performed to discriminate leucospermia with immature germ cells and leucocyte like cells. The cellular painting between yellow to brown showed the peroxidase positive leucocyte, pink cellular painting showed other cells (immature germ cells). Sperm DNA damage was determined with Halo sperm test which is a sperm chromatin dispertion (SCD) test.
Main results and the role of chance: Mean age of the control group and leucospermia group was 33.2 and 31.5 years, and total sperm count was 83.5×10^6/ml and 78.7 ×/ml, respectively. There were no statistically differences between ages and total sperm counts. Sperm DNA fragmantation index (DFI) was 8% in control semen and 47.5% in leucospermic semen which showed statistically difference (p value 0.001).
Limitations, reasons for caution: In this study, only leucospermic men and who had normal semen parameters were included. The men who had oligozoospermia, azoospermia, or endocrine disorders were excluded.
Wider implications of the findings: It has been showed that sperm DNA damage was significantly higher in couples who had unsuccessfull IUI. One of the main factors in male infertility etiology is urogenital infections. According to

WHO, ≥ 1×10^6/ml leucocyte in semen is abnormal. Leucospermia has toxic effects on sperm and may cause infertility.
Trial registration number: This is not a clinic trial.

P-033 Human prolactin inducible protein in seminal plasma as a marker of azoospermia

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Study question: Is it possible to use concentration of prolactin inducible protein in seminal plasma as a marker of azoospermia ?
Summary answer: Our results showed that the seminal plasma samples from azoospermic donors was significantly reduced levels of protein PIP compared to healthy donors.
What is known already: Prolactin inducible protein (PIP) is expressed in most organs that contribute to human body fluids. PIP binds to many proteins and play important roles in many important biological processes such as fertility or immunoregulation. PIP is represented in high concentration in seminal plasma, where protect the spermatozoa from the damage by IgG. Recently, in seminal plasma was isolated complex of PIP, with human serum albumin. Albumin has been known to preserve the motility of sperm, therefore, this native albumin-PIP complex in human seminal plasma may suggest another important role of PIP, which can directly be correlated with male fertility/infertility.
Study design, size, duration: We developed a novel enzyme immunoassay for quantitative measurement of human PIP suitable for detection of PIP in seminal plasma. During 1 year we measured PIP in seminal plasma from donors with different diagnoses (normozoospermia 24 patients, azoospermia 13 patients, asthenospermia 19 patients, oligospermia 23 patients and after vasectomy 2 patients).
Participants/materials, setting, methods: We assembled sandwich ELISA for the quantitative measurement of huma PIP in seminal plasma This ELISA test is based on two sheep specific polyclonal anti-human PIP antibodies. The Standard used in this kit is native PIP protein isolated from human seminal plasma. Before PIP analyzes we performed evaluation of spermiogram in each donor. In this study were only men between 23–45 years old.
Main results and the role of chance: In the seminal plasma samples from azoospermic patients was determined significantly lower concentrations of PIP (709.02 µg/ml) compared with a seminal plasma samples from normozoospermic donors (1366.09 µg/ml), asthenozoospermic donors (1660.84.09 µg/ml) and also oligozoospermic donors (1378.87 µg/ml) ($P < 0.05$). Indeed concentration of PIP in donors after vasectomy were similar (1544.98 µg/ml) as in control normospermic donors (for low number of samples we cannot perform correct statistical evaluation).
Limitations, reasons for caution: For future development of this diagnostic method is necessary analyse more samples from men after vasectomy. Higher number of samples from this group is necessary for correct evaluation and final decision regarding obstructive or nonobstructive azoospermy.
Wider implications of the findings: On the basis of these results we can conclude that samples from azoospermic donors was significantly reduced levels of protein PIP compared to healthy donors. ELISA for the quantitative measurement of human PIP in seminal plasma could be used as a promising non-invasive method for the diagnosis of azoospermia.
Trial registration number: without trial registration number.

P-034 Physical activity is correlated with sperm quality in sperm donors

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Study question: Ascertain the influence of physical activity in sperm quality
Summary answer: Higher levels of intense physical activity was associated with better semen parameters
What is known already: The literature presents conflicting results describing the absence of relationship, beneficial or negative effect on the relationship between physical activity and semen quality and other markers of testicular function. The ideal level of physical activity is unknown from the point of view of the seminal quality. Therefore from a practical point of view, there are no arguments when advising males within couples with fertility problems to change their physical activity
Study design, size, duration: Prospective observational non randomized in order to determine influence of one variable on a given outcome
4 cohort groups of physical activity are established, according to their score in IPAQ: **Very high,** total minimum of physical activity of al least 3000 MET week: 26 donors
High, minimum of physical activity between 1500–3000 MET week: 46 donors
Moderate: minimum of physical activity between 600–1500 MET week: 9 donors
Low: until < 600 MET week: 4 donors
Participants/materials, setting, methods: 85 Candidates for sperm donors < 35 years old in the donation program at IVI Bilbao during 2015–2016
International Physical Activity Questionnaire (IPAQ) was offered before sperm donation. Energy requirements defined in METs being multiples of the metabolic rate that quantifying the phisical activity assigned according each activity

Semen parameters of the donors to study by means of a spermiogram were evaluated according to WHO 2010, without knowing IPAQ questionnaire data
ANOVA and linear regression analysis were performed
Main results and the role of chance: Ejaculated volume did not differ significantly between the 4 groups although it was slightly lower in the group of low activity: (2.6 ± 1.60, 2.8 ± 0.93, 3.4 ± 1.53, 3.5 ± 1.6).
Concerning sperm concentration/ml, it was higher in the group of intense activity without statistical significance. (52 ± 41.94, 40 ± 28.82, 34.71 ± 54, 53 ± 39.7) was not statistically significant.

Something similar happened to the **total number of spermatozoa**, groups higher in high and very high again without statistical significance. (120 ± 89.81, 110 ± 78.13, 115.02 ± 170, 170 ± 142.31)

Concerning the **total number of progressive** mobile spermatozoa, there is a higher average of progressive ones (51 ± 36.19, 49.94 ± 62, 81 ± 63.12, 100 ± 89.28)

Among the total concentration of sperm and the highest values of IPAQ a certain trend in the linear regression analysis were observed ($r = 0.016$, $p = 0.24$) despite the p value, which doesn't become significantly statistical

A similar pattern was observed with the total progressive mobile spermatozoa when physical activity increased, expressing a high IPAQ value ($r = 0.045$, $p = 0.05$)

Physical activity was not related with the sperm morphology
Limitations, reasons for caution: The small size of the population sample goes against the correction of the confounding factors. The lack of significant differences between the averages which is likely due to the small sample size and be unbalanced groups. The results may not be the same in older populations. IPAQ is universally accepted questionnaire.
Wider implications of the findings: In this study, the intense physical activity range not only had no adverse effects on semen quality, but also was associated with better semen parameters.

While more studies are needed, one could speculate with infertile male suitability of increasing their physical activity
Trial registration number: NCT02683057.

P-035 Clinical pregnancy rates comparing different sources of spermatozoa

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Study question: Compare pregnancy and fertilization rates among the different sources of spermatozoa (ejaculate, epididymis, and testicle).
Summary answer: Our results confirm that there is no difference in pregnancy and fertilization rates when comparing the different sources of spermatozoa (ejaculate, epididymis, and testicle).

What is known already: It is known that the spermatozoon is considered mature after contacting the ducts of the epididymis, before ejaculation. However, several studies have shown that spermatozoa collected directly from the testicle and the epididymis can fertilize and produce completely normal embryos. Meantime there is an ongoing debate whether the source of sperm cells has influence on the outcome of ICSI cycles.
Study design, size, duration: Retrospective cohort study between 2000 and 2015.
Participants/materials, setting, methods: There were performed 6508 cycles of ICSI. The couples were divided into three groups: epididymis, testicle and ejaculated. Each one with 282, 282, and 5944 cycles, respectively. Eighteen hours after insemination by ICSI, fertilization was verified. All the embryos were kept in culture until transfer at the cleavage or blastocyst stage. Twenty one days after the transfer, the clinical pregnancy was confirmed by ultrasound. Fertilization and pregnancy rates were compared using the chi-square test.
Main results and the role of chance: A total of 45258 eggs were inseminated, 2335 were inseminated with sperm from the epididymis; 2673 oocytes with testicular sperm and 30896 inseminated with spermatozoa from ejaculate. The fertilization rate was statistically higher in patients who used ejaculated spermatozoa (76.8%), followed by the group in the epididymis (63%), and testis group provided the lower fertilization rate (57.6%) (p <0.05). Pregnancy rates were similar in the 3 groups; 49% (epididymis), 45.1% (ejaculate) and 43.1% (testicle), with no statistical difference (p.0.05). As well as the clinical pregnancy rate 40.3% (epididymis), 39.1% (ejaculate) and 34.7% (testicle) also did not show statistical significance. Although the fertilization rate is higher in the ejaculated group, pregnancy rates were not different between then. Thus, the source of the sperm does not influence the outcome of IVF.
Limitations, reasons for caution: Although there were a very different number of cycles among the groups, this did not affect the results found in this study.
Wider implications of the findings: The source of spermatozoa does not affect the pregnancy rate outcome.
Trial registration number: Not necessary.

P-036 The testicular histopathology as a predictive factor of sperm retrieval in patients with non-obstructive azoospermia (NOA)

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Study question: The purpose was to investigate the sperm retrieval rate and sperm recovery time after testicular biopsy, and clinical outcomes according to five different testicular histopathologies.
Summary answer: Testicular histopathology is predictive of research time needed for sperm retrieval. No difference was found in clinical outcomes in ICSI cycles performed with testicular sperm.
What is known already: Azoospermia occurs in 10–15% of infertile male population. Non-obstructive azoospermia (NOA) is caused by testicular failures and represents 60% of azoospermia. It is due to testicular or pre-testicular damages, such as hypothalamus pituitary dysfunctions. Surgical retrieval of spermatozoa in order to perform an ICSI cycle, is the only possibility of fathering a child in patients suffering for NOA. Some studies show that testicular histology is the best predictor of success in sperm retrieval from testicular biopsy, such as Testicular Sperm Extraction (TESE).
Study design, size, duration: This retrospective study was performed from March 2005 to November 2015 on 518 patients. Patients were divided in five different groups, based on different testicular histopathology: Sertoli-cell-only (A), maturation arrest (B), severe hypo-spermatogenesis (C), normal spermatogenesis (D), Sclerohyalinosis (E). Sperm recovery time (SRT), fertilization rate (FR), clinical pregnancy (CPR) and live birth rates (LBR) for each enrolled groups, were analyzed. Statistical analyses were performed using student T-test and Fisher's exact test (P value < 0.05).
Participants/materials, setting, methods: If spermatozoa were not retrieved in one testis, the biopsy was performed on the other one. In each surgery, a small tissue sample was histologically analyzed. For each Group the same observation time, from few minutes to almost 12 hours, was applied. Recovered spermatozoa were used for fresh ICSI of partner's oocytes or cryopreserved for future use. There were no differences in male and female mean ages among the five groups.
Main results and the role of chance: Patients who underwent conventional TESE were 518: 200, 94, 73, 140, 11 in groups A-B-C-D-E, respectively. Spermatozoa were recovered in 348 patients (67.2%): 64 (32%), 77 (81.9%), 63 (86.3%), 135 (96.4%), 9 (81.8%) in groups A-B-C-D-E, respectively. Statistical difference in sperm retrieval rate, was observed in group A compared to all other groups ($p \leq 0.0001$) and in group D compared with groups B, C, E ($p \leq 0.002$). For each group, the mean of SRT was considered: 3.4, 2.7, 2.5, 1.0, 3.3 hours in groups A-B-C-D-E, respectively. Statistical significant difference in SRT was found only among group-D compared to all the other groups ($p \leq 0.02$). A total of 336 (A-64, B-77, C-63, D-123, E-9) patients with positive spermatozoa retrieval performed an ICSI cycle, 119 using fresh and 217 frozen spermatozoa. Injected oocytes were 2027. FR was 58.3% (224/384), 54.3% (266/490), 61.5% (221/359), 67.3% (504/749), 48.9% (22/45) in groups A-B-C-D-E, respectively and a statistical difference was found in group-D compared with A, E and B ($p \leq 0.01$) and in group-B compared with C ($p = 0.03$). CPR was 36.9% (17/46), 32.8% (20/61), 30.6% (15/49), 34.1% (31/91), 25% (1/4) in groups A-B-C-D-E, respectively (NS). LBR was 14.3% (15/105), 10.6% (15/142), 9.2% (11/120), 19.4% (41/211), 10% (1/10) in groups A-B-C-D-E, respectively (NS).
Limitations, reasons for caution: Clinical data are not complete since many patients with positive spermatozoa retrieval didn't undergo an ICSI cycle. Sperm recovery time is strictly related to the number of biologists involved in the research and to the laboratory equipment. Performing a multiple-TESE or a micro-dissection TESE could shorten sperm recovery time.
Wider implications of the findings: Sperm retrieval research time could be a limiting factor for ICSI procedure, related to in-vitro oocyte ageing. Testicular histopathology could give valuable information with regard to the likelihood of retrieving sperm in NOA patients. More severe is the pathology and a longer time is necessary to retrieve spermatozoa.
Trial registration number: Not applicable.

P-037 Pathological threshold of human sperm DNA oxidation, an increase value for infertility diagnostics

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Study question: What is the pathological threshold of human sperm DNA oxidation concerning men infertility diagnosis? What is its real impact on human sperm quality?
Summary answer: Our findings determined a pathological threshold of human sperm DNA oxidation related with sperm parameters associating intensity mean of fluorescence and percentages of oxidized spermatozoa.
What is known already: Oxidative stress plays a major role in male infertility by inducing sperm DNA damage. Sperm nuclear integrity is essential to conduct normal embryonic development and paternal genome transmission to offspring. Therefore it is essential to provide a test permitting to measure sperm DNA alterations.

8-OHdG is the major adduct found in human sperm due to oxidative stress. Nevertheless, there is no standardized and consensual protocol to measure 8-OHdG in human sperm in clinical practice. We previously validated an immuno-detection protocol for sperm 8-OHdG biomarker and measured its accuracy, reliability, sensibility and specificity in comparison with a commercial kit "OxyDNA assay®".
Study design, size, duration: In this prospective study, sperm DNA oxidation level of 80 patients (35 ± 1 years) were measured during 13 months. Sperm DNA oxidation level was simultaneously measured using the commercial "OxyDNA assay®" kit and our validated 8-OHdG immuno-detection protocol. DNA oxidation level obtained with both methods was confronted with semen parameters and patients characteristics.

Participants/materials, setting, methods: Forty patients were normozoospermic, 40 presented abnormal sperm parameters (leuco-, necro-, astheno and teratozoospermia). Sperm DNA oxidation was assayed with a previously validated immuno-detection protocol using anti-8-OHdG primary antibody and Alexa 488-conjugated secondary antibody. Sperm 8-OHdG measured with "OxyDNA assay®" and immuno-detection were detected by flow cytometry (FCM) computing quantification and qualification of sperm DNA oxidation using fluorescent spermatozoa percentage and oxidation intensity mean of fluorescence.

Main results and the role of chance: Using immuno-detection protocol, we measured significant difference in 8-OHdG spermatozoa percentage or/and in intensity mean of fluorescence for all measured sperm parameters, i.e., necrozoospermia ($n = 8$, $p < 0.05$), asthenozoospermia ($n = 12$, $p < 0.01$), teratozoospermia ($n = 27$, $p < 0.05$) and leucozoospermia ($n = 14$, $p < 0.001$) patients in comparison with respective normal groups.

Overweight patients ($n = 20$, 1478 ± 373, $p < 0.01$) showed also a significant higher mean of fluorescence *versus (vs.)* normal weight group ($n = 14$, mean: 379 ± 75).

Significant correlations were observed between sperm DNA oxidation fluorescence intensity and body mass index ($r = 0.6$, $p < 0.001$), sperm concentration ($r = 0.30$, $p < 0.05$), round cells concentration ($r = 0.26$, $p < 0.05$) and the polynucler neutrophil concentration ($r = 0.5$, $p < 0.001$).

Using OxyDNA assay®, only teratozoospermia group presented a significant lower percentage of 8-OHdG spermatozoa ($p < 0.01$) vs. normal group.

The validated immuno-detection protocol allowed to determinate a human sperm DNA 8-OHdG pathological threshold associating intensity mean of fluorescence and percentage of oxidized spermatozoa. Patient having higher values (sperm percentage and fluorescence intensity) than the determinated threshold presented mainly leucospermia or/and asthenozoospermia, confirming the diagnostic value of the biomarker.

The distribution of marked sperm cells values using OxyDNA assay® didn't show any threshold using either oxidated sperm percentage or fluorescence intensity.

Limitations, reasons for caution: Oligozoospermia population was not evaluated in the study.

Wider implications of the findings: The present study is the first to determine a pathological threshold concerning human sperm DNA oxidation, related with sperm parameters notably leucozoospermia and asthenozoospermia.

This 8-OHdG immuno-detection protocol, preliminary validated for clinical practice, will improve male infertility diagnosis and patient support notably with an adapted and effective antioxidants oral supplementation.

Trial registration number: None.

P-038 The influence of high values of sperm DNA fragmentation on the quality and genotype of the embryos

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Study question: Whether if the high values of sperm DNA fragmentation effect quality and genotypes of the embryos in PGS programs.

Summary answer: The positive correlation was found between the sperm DNA fragmentation and the high frequencies of aneuploidy and deletions in the embryonic genome.

What is known already: Many authors agree that early spontaneous abortions might be associated with the high level of DNA damage (DNA fragmentation). FISH analysis of several chromosomes has shown that patients with high values of sperm DNA fragmentation have a high frequency of chromosomal abnormalities in spermatozoa's genomes. We find this problem of high interest and believe that it needs in-depth study using the contemporary methods of whole-genome embryo screening.

Study design, size, duration: The retrospective analysis of 170 couples, undergoing the IVF treatment with the evaluation of functional semen parameters (concentration, motility, morphology), and sperm DNA fragmentation. The rates of fertilization and blastocyst formation were evaluated. The frequencies of autosomal monosomies and trisomies and disomies in sex chromosomes, as well as the number of deletions and translocations in the embryonic genomes, were evaluated using the whole-genome screening by CGH.

Participants/materials, setting, methods: ICSI+PGS program, fertilization and embryo culture according to the manufacturers recommendations of ORIGIO (Denmark), sperm DNA fragmentation was evaluated using a TUNEL assay (the normal level of DNA fragmentation is < 15%), array CGH (Agilent, USA) was used for 24-chromosome embryonic genome analysis.

Main results and the role of chance: Our results show that there are lower rates of blastocyst formation (24% vs. 55%, $p < 0.05$) in the group with high values of sperm DNA fragmentation in comparison with the group with normal values of this parameter. PGS has shown the significantly lower number of embryos with normal genotype (suitable for transfer) (42.9 vs. 58.4 $p < 0.0095$) and the increase of frequencies of deletions and large translocations in embryonic genomes (40.95 vs. 6.14 $p < 0.0095$) for the couples with high values of DNA fragmentation which is caused by the transmission of genetic damages from the nuclei in a spermatozoa to the genome of an embryo. Higher frequencies of trisomies (21.04 vs. 19.43 $p < 0.005$) and autosomal monosomies (18.69 vs. 17.59 $p < 0.05$) were found in our group of study. Damaged or abnormal spermatozoa very often carry an extra sex chromosome, which leads to the birth of a child with the Klinefelter syndrome (47, XYY; 48, XXXY etc.). In the group with high values of DNA sperm fragmentation, we found higher frequencies of disomies in sex chromosomes in comparison with the group with normal values of this parameter (11.58 vs. 7.35 $p < 0.0095$).

Limitations, reasons for caution: Our study didn't include a large number of couples. It might be reasonable to carry on a study in which values of sperm DNA fragmentation before and after antioxidative treatment are compared as we expect to find better results of the genetic screening after treatment.

Wider implications of the findings: Our study shows the correlation between the high values of sperm DNA fragmentation and deletions in the embryonic genome as well as autosomal aneuploidies and disomies in sex chromosomes. Nevertheless, we highly recommend to undergo PGS for the couples with high values of sperm DNA fragmentation.

Trial registration number: N/A.

P-039 Toll like receptors signaling pathway PCR array in recurrent implantaion failure patient with high and low sperm DNA damage

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Study question: What are the differences between Toll like receptor signalling pathway PCR array in recurrent implantation failure (RIF) patients with high sperm DNA damage and low sperm DNA damage?

Summary answer: TLRs, especially TLR5, its signalling pathways and inflammatory cytokines have higher expression in RIF couples with high DF, compared with RIF ones with normal DF.

What is known already: TLRs are the major compartment of innate immune system. It is well established that microbial PAMPs are ligands for TLRs. However, it is becoming clearer that certain locally produced endogenous substances can also stimulate TLRs. Also, TLRs play a role in pathogenesis of unexplained infertile couples with high DF (DNA fragmentation) and Sperm DNA damage plays an important role in immunological interaction of sperm with female reproductive tract.

Study design, size, duration: 30 Fresh semen samples were obtained from RIF couples in two groups.1. fiftheen samples with DNA fragmentation more than 30% (by SCSA assay) and 2. fiftheen with less than 5%. All these semen samples, after washing were co-incubated with human fallopian tube cell line (OE-E6/E7) in triplicate for 24 hours.

Participants/materials, setting, methods: TLRs genes and protein expression in OE-E6/E7 cell line was investigated By RT-PCR and Immunostaining respectively and compared with human fallopian tube tissue. After co-incubation of sperm in both group with fallopian tube cell line, the profile of Toll like receptors signalling pathways gene expression were evaluated by TLR PCR array kit in OE-E6/E7 cells.

Main results and the role of chance: TLR1-6 gene and protein were expressed in OE-E6/E7, like fallopian tube tissue. The mean relative expression of TLRs, especiallyTLR5 were higher significantly in response to sperm with high DNA

fragmentation in compared to sperm with low DNA fragmentation ($P<0.05$). Also, MYD88 dependent pathway had higher expression compared with MYD 88-independent pathway. Besides, the vast majority of adaptors, effectors and member of NFkB, Jak/stat and cytokine mediated signaling pathway were intermediately to highly expressed in RIF patient with high DF than low one.

Limitations, reasons for caution: The results need to be confirmed in more cases.

Wider implications of the findings: DAMP of TLRs causes TLRs signalling activation. No DAMP for TLR5 have been recognized yet, but several studies showed the role of TLR5 in some disease without a role for its specific PAMP. So, DNA damage should be considered in treatment of RIF due to high production of inflammation.

Trial registration number: N/A.

P-040 Does the number of veins ligated during microsurgical subinguinal varicocelectomy influence semen and hormone outcome?

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Study question: The study of factors predicting semen improvement after varicocele ligation is motivating. in this study we ask whether the number of veins ligated during surgery play any significant role.

Summary answer: A significant improvement in semen parameters occurs after microsurgical varicocelectomy, this improvement does not seem to be related to the extent of vein ligation.

What is known already: The subject has been evaluated previously in 2 studies showing a superior outcome in semen parameters with higher number of vein ligation. However, both studies were small sized and the surgical method was not uniform in one of them. Newer varicocele ultrasound grading systems consider the presence of a venous plexus of veins and the sum of diameters of all detected veins in grading. Although the number of veins witnessed during surgery can vary greatly irrespective of the clinical grade, their value in the pathophysiology and/or the detrimental effects of varicocele on testicular function is not known.

Study design, size, duration: This is a retrospective study of 682 records of patients undergoing left microsurgical varicocelectomy between 2007 and 2014. Patients presenting with infertility were included in this study. While the exclusion criteria were patients who underwent surgery for an indication other than infertility, patients with prior history of varicocele ligation, presence of abnormal genetics as cause of infertility, history of mumps orcitis and history of medical treatment before varicocelectomy.

Participants/materials, setting, methods: A total of 378 records were included. Semen and hormone test results done initially were compared to those performed 9 months after surgery. Patients were divided into 2 groups according to their initial semen results; abnormal (sperm count < 20 million/ml and/or total motility <50% and/or abnormal morphology >70%) and normal groups. Patients were also subdivided depending on the number of veins ligated during surgery into 3 subgroups; < 5, 5–10 and > 10 veins.

Main results and the role of chance: Overall, sperm count, total motility and progressive motility were significantly increased in 62%, 60.3% and 53.3% of patients postoperatively (p=0.001). No significant differences in hormone levels were detected. 332 patients had an abnormal semen analysis while the remaining 46 patients had a normal result. Sperm count, total motility and progressive motility were significantly increased after varicocelectomy, only in patients with abnormal initial semen analysis (p=0.001). Less than 5 veins, 5–10 veins and >10 veins were ligated in 23%, 28.3% and 48.7% of patients, respectively. A correlation between preoperative semen and hormone levels with the number of veins ligated intraoperatively did not reveal any statistically significant deference. Moreover, the extent of vein ligation did not have any statistically significant effect on the percentage improvement in semen and hormone levels between both groups.

Limitations, reasons for caution: Sperm chromatin integrity was not assessed in this study and can be considered as a limitation due to its vital relation with varicocele. Furthermore, no comparison was performed between ultrasound and intraoperative findings, since in most patients the ultrasound sis not have details about the number of veins detected.

Wider implications of the findings: This study disagrees with previous smaller reports, which suggested an influence for the number of ligated veins on surgery outcome. Varicocele imaging with ultrasonography is an addition to the clinician's armamentarium, however it does not replace physical examination in his clinical judgments.

Trial registration number: (NA) This is a retrospective study.

P-041 Spermatozoa with both abnormal chromatin condensation and large nuclear vacuoles seem to have a negative influence on clinical outcomes, in oocytes-donation cycles with repeated failures

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Study question: Find a correlation between live birth rate and sperm quality, valuated according to MSOME (Motile sperm organelle morphology examination) criteria, in oocytes-donation (OD) cycles.

Summary answer: We suppose a negative effect of spermatozoa with both large nuclear vacuoles (type IV) and abnormal chromatin condensation, in clinical outcomes, in oocytes-donation cycles.

What is known already: During spermiogenesis, the somatic histones are substituted by protamines for a normal nuclear chromatin condensation. Aniline blue selectively targets lysine-rich histones showing blue nuclei typical of spermatozoa with abnormal condensation. An association between male infertility and the anomalous chromatin condensation, are reported. Also morphological abnormalities of sperm head linked to the number and to the dimension of nuclear vacuoles, are well-known. In fact, there are evidences that confirm the relationship between the high-magnification sperm selection and both the number of blastocysts obtained and the clinical outcomes.

Study design, size, duration: Fresh semen samples of sixteen patients, of whom spermatozoa were beforehand used for oocytes-donation cycles with repeated *in vitro* failures, in our and in other laboratories, were successively analyzed, for their nuclear vacuoles according to MSOME criteria. Same samples were used for aniline blue staining and FISH (Fluorescence In Situ Hybridization) analysis. Samples were collected from March to December 2015. The man mean age was 43.3 ± 6.2. Statistical analyses were performed using student-test (P value <0.05).

Participants/materials, setting, methods: Samples, not used for ICSI, were examined for seminal parameters according to WHO-2010 criteria. Sperm morphology was evaluated allowing to MSOME criteria identifying four classes: type I-II with none or one small vacuoles and normal head shape; type III with plus three vacuoles and normal head shape; type IV with large vacuoles and abnormal head shape. Aniline blue (v.n. <30) and FISH analysis (13, 15, 16, 17, 18, 21, 22, X, Y chromosomes) were performed.

Main results and the role of chance: The sixteen semen samples analyzed (concentration 40.1 ± 18.3 M/ml and progressive motility 44.4 ± 16μm/sec), appear to have normal ploidy for the nine investigated chromosomes by FISH analysis. For each patient, 100 spermatozoa for MSOME and 100 for aniline blue, were analyzed. Total sperm examined, using MSOME criteria, were divided as follow: 588 type I-II, 412 type III, 600 type IV. In nine patients, 321 spermatozoa showed positivity to aniline blue staining.

Analyzing data, we find a positive correlation between semen samples with more type IV spermatozoa and the aniline blue positivity ($P < 0.0001$). At the same time there is a negative correlation between type I-II sperm number and the aniline blue positivity ($P = 0.001$). Finally, a statistical significance relationship between the type III sperm and the aniline blue ($P = 0.0145$), was detected.

Generally, the OD cycles with worst clinical outcomes (embryo degenerations, implantation failures, abortions) were performed with semen sample successively resulted with more vacuoles in spermatozoa (type IV) and showing blue nuclei typical of an abnormal chromatin condensation.

Limitations, reasons for caution: The main limitation of this study is the low number of samples, due to the impossibility, in some cases, to recover the fresh semen samples after the OD cycles failures. More samples should be analyzed, in the future, to reinforce our conclusions.

Wider implications of the findings: Our data show that the sperm selection, with high magnification microscopy, could be useful to improve the ICSI successful in OD cycles with repeated failures in clinical outcomes.

Trial registration number: Not applicable.

P-042 Human seminal plasma of different men induce different power of uterine contractility in a uterus perfusion model

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Study question: Does human seminal plasma of different men induce different contraction strength of the uterus?
Summary answer: Application of individual seminal plasma into the porcine uterus cause different reaction of uteri regarding the contractility (power, frequency, AUC). Data suggest a dose-dependent effect.
What is known already: A sufficient contractility of the uterus plays an important role for successful conception. Several substances as oxytocin, estradiol or prostaglandins are known to support uterine contractility. The fact that these substances are ingredients of the seminal plasma stresses its relevance for the reproductive function. Examinations in animals as well as in humans showed seminal plasma`s impact for conception. However seminal plasma composition and reciprocity of the substances seem to be very complex and not completely understood yet. No studies about the dose-dependent effect of seminal plasma application into the whole uterus have been published.
Study design, size, duration: The aim of this experimental in-vitro study was to evaluate the influence of human seminal plasma on their contractility by using an extracorporal perfused pig uteri. Ejaculate of 36 men with normozoospermia were taken from the laboratory of the university hospital Erlangen. The ejaculates were injected into the perfused uterus of 54 pigs to analyze whether individual dose-dependent effect of seminal plasma on the uterine motility exists by repetitive intraluminal bolus application of different volumes.
Participants/materials, setting, methods: The uteri were stored in a special nutrition solution and perfusion was managed with the aid of a 24 G canula inserted into both Arteriae uterinae. Uterine pressure as the area under the curve (AUC) was measured by a precision pressure catheter with two measure points near to the ovaries (IUP 1) and near to the cervix (IUP 2) at four time points (t0: minute 0–60; t1: minute 61–105; t2: minute 106–150; t3: minute 151–195).
Main results and the role of chance: Injection of 0.3, 0.6 and 1.2 ml seminal plasma increased uterine pressure significantly. It was not significantly higher at IUP 2 compared with IUP 1. Contraction frequency improved after injection of 0.3 and 0.6 ml seminal plasma. The ovarian and the cervical measure point showed no significant difference concerning maximal and average pressure amplitude after application of 0.3 ml seminal plasma, but revealed significantly higher values at IUP 2 after administration of 0.6 and 1.2 ml seminal plasma. The seminal plasma application into the porcine uterus significantly increases contractility in form of AUC, contraction frequency as well as average and maximal values of the contraction amplitude. The data suggest a dose-dependent effect. The direct comparison of the integrals of intrauterine pressure over time after administration of seminal plasma of different men showed a high variation between 3769 and 2132 mmHg/h.
Limitations, reasons for caution: Physical disturbance variables potentially influencing the results were limited as much as possible. For preventing an increase in uterine contractility because of the temperature seminal plasma was warmed up to the nutrition solution temperature. With a slow uniform injection a steady seminal plasma distribution and a low pressure were reached.
Wider implications of the findings: The results demonstrate the importance of physiologic seminal plasma composition and introduce new potential options in diagnostic and treatment for couples with an unfulfilled wish for a child. Further investigations about the exact seminal plasma composition and the interaction of the ingredients are necessary.
Trial registration number: None.

P-043 Calretinin increases androgen production in testicular Leydig cells

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Study question: In the present study, we explored the effect of calretinin (CALB2) on the regulation of androgen production in testicular Leydig cells and its potential mechanism associated with Ca^{2+} pathway.
Summary answer: CALB2 plays a positive role in the acceleration of androgen production in Leydig cells by the Ca^{2+} pathway, besides PKC and PKA pathways.
What is known already: The Ca^{2+}-binding protein CALB2 is well known as the Ca^{2+}-buffering function, primarily expressed in specific neurons, while it can also function as the Ca^{2+} sensor. And it emerges as a multi-functional protein also associated with development, i.e., cell proliferation, differentiation and cell death recently. Ca^{2+}, as the second messenger, participates in hormonal synthesis which could be regulated by PKA and PKC signals. Previously we observed CALB2 was also expressed in testicular Leydig cells and had a positive correlation with androgen levels, suggesting CALB2 could participate in regulating steroidogenesis.
Study design, size, duration: The change of androgen production was detected in the overexpressed and downexpressed-CALB2 Leydig cells. Then these cells were treated by PKC inhibitors (Chelergthrin), PKC and PKA inhibitors (Daphnetin) singularly or combining with hCG, in order to clarify the steroidogenensis regulated by CALB2.
Participants/materials, setting, methods: The cultured R2C and MLTC-1 were transfected by LV-SiRNA-Calb2 and LV-Calb2, respectively. The transfected MLTC-1 were treated with 0.1 IU/mL hCG for 4 h. All medium was collected for measuring androgen via radioimmunaossay, Ca^{2+} levels were detected by the confocal microscopy with calcium fluorescent probes X-Rhod-1,and Western blotting was used to examine CALB2 expression. After transfected MLTC-1 were treated by Chelergthrin, Daphnetin, and calcium releasing agents (Clopiazonic) with or without 0.1IU/mL hCG for 4h, androgen was measured.
Main results and the role of chance: Steroidogenesis was significantly increased in the hCG treated cells, while expression of calretinin increased ($P < 0.01$). Progesterone production in the R2C cells with LV-SiRNA-Calb2 was decreased ($P < 0.05$), and testosterone production in the MLTC-1 cells with LV-Calb2 was significantly increased ($P < 0.05$). In the LV-Calb2 transfected MLTC-1 cells with or without HCG, the cytoplasmic Ca^{2+} was up-regulated compared with the corresponding vector groups ($P < 0.01$). However the cytoplasmic Ca^{2+} was decreased ($P < 0.05$) in the R2C cells with LV-SiRNA-Calb2. In the LV-Calb2 transfected MLTC-1 cells with or without HCG, testosterone production was significantly induced by clopiazonic ($P < 0.01$), while daphnetin and Chelergthrin had no impact on testosterone production compared with the vectors ($P > 0.05$).
Limitations, reasons for caution: From our study, we know CALB2 enhances Ca^{2+} to promote steroidogenesis in Leydig cells. However, overload of Ca^{2+} may injury Leydig cells, so that we should quantify Ca^{2+} in fear of Ca^{2+} overload.
Wider implications of the findings: This study demonstrates that Calretinin is involved in the regulation of testicular androgen synthesis, so that it enhances our knowledge on the intracellular signaling mechanisms of testosterone synthsis, and provides us a theoretical basis and clinical understanding for male reproductive endocrine disorders and the late-onset hypogonadism.
Trial registration number: –

P-044 Clinical outcome in intracytoplamic sperm injection (ICSI) cycles using testicular sperm in post chemotherapy non-obstructive azoospermia (NOA)

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Study question: What are sperm retrieval rates (SRR) by microdissection testicular sperm extraction (micro TESE), fertilization rate, and embryonic development among post chemotherapy NOA couples?
Summary answer: The SRR in post chemotherapy NOA was higher than that of patients with other uneplained NOA patients, and better fertilization and embryonic development were achieved.
What is known already: The survival rates for pediatric and adolescent patients with various types of malignancies have dramatically improved with advances in chemotherapeutic treatments. In male cancer survivors, the restoration of fertility and achievement of paternity have become important concerns. The recent success of micro TESE combined with ICSI for patients with NOA indicates that ART (assisted reproductive technologies) offer a potential new treatment option for affected couples and so that this procedure provides hope for men of reproductive age who have not undergone sperm cryopreservation before chemotherapy.
Study design, size, duration: We performed a retrospective study based on a reproduction center in Japan and evaluated 26 post chemotherapy NOA patinets (including 6 patients with bone marrow transplantation (BMT)) and 250 NOA

patinets with 46XY without past history (unexplained NOA; not including after orchidopexy, Klinefelter syndrome, cryptozoospermia, mumps orchitis, etc) who underwent micro TESE by a single surgeon (T.I.) between September 2013 and December 2015.

Participants/materials, setting, methods: The cancer types included testicular cancer, colon cancer, Hodgkin's lymphoma, non-Hodgkin's lymphoma, leukemia, neuroblastoma, and osteosarcoma. The age at micro TESE and chemotherapy was 34.6 ± 9.0 and 16.9 ± 7.9 years, respectively, and the mean wives age at ICSI was 34.0 years. Two pronuclei (2PN) oocytes, blastocysts development, good-quality blastocysts (Grade >3BB by the Gardner score), biochemical and clinical pregnancies rates were examined. Statistical analysis was performed using unpaired t-tests and chi-squared tests.

Main results and the role of chance: None of the patients had undergone cryopreservation of their sperm before chemotherapy. SRR in post chemotherapy NOA (13/26 = 50.0%) was higher than unexplained NOA (73/250 = 29.2%) patients ($p < 0.05$) in whom micro-TESE was performed. Three of 6 post BMT patients were retrieved spermatozoa. Two of 3 patients even showed 46XX (transplantation from women). The 13 patients who failed to obtain sperm could not find any germ cells in their testicular samples during micro TESE and histopathological findings (Sertoli cell only syndrome). With respect of type of cancer, there was no predictor for SRR and no significant differences in the pregnancy and live birth rates. In sperm retrieved post chemotherapy NOA, age at chemotherapy end was older (22.0 ± 8.3 years) than failure group (11.8 ± 7.7 years) ($p < 0.01$). 2PN oocytes, blastocysts development, and good-quality blastocysts rates were 64.9%, 50.6%, and 51.3% in post chemotherapy NOA and 60.1%, 44.5%, and 41.9% in unexplained NOA. Biochemical pregnancy and clinical pregnancy rates per embryo transfer (ET) were 57.1% (12/21) and 47.6% (10/21) in post chemotherapy NOA, and 32.8% (44/134) and 25.4% (34/134) in unexplained NOA, respectively. Clinical pregnancy rate per patients was 75.0% (9/12) in post chemotherapy NOA and 58.5% (31/53) in unexplained NOA.

Limitations, reasons for caution: The participants are limited to the NOA but not cryptozoospermic patients. No hormonal treatment was performed before micro TESE. There was a lack of standardization with respect to the chemotherapeutic regimen. The safety and screening for cancer and congenital malformations among these children has not been fully investigated.

Wider implications of the findings: The patients with post chemotherapy NOA no longer should be considered sterile without performing micro TESE-ICSI. It is useful to obtain reliable embryonic information from NOA patients and to encourage micro TESE for post chemotherapy NOA patients even with severe dysregulation of spermatogenesis.

Trial registration number: N/A.

P-045 The application of 3-methyladenine with lithium chloride increases the detrimental effects of lithium chloride on sperm number and motility

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Study question: Does 3-methyladenine (3-MA) prevent detrimental effects of lithium chloride (LiCl) on sperm number and motility at normospermic males?

Summary answer: The application of 3-MA with LiCl slightly increased the detrimental effects of lithium chloride on sperm number and motility at normospermic males.

What is known already: Chemical LiCl with its' anti-physicotic and anti-neoplastic effects is accepted as a drug and it has been being used used in clinic for 60 years. The detrimental effect of LiCl on male infetility through the inhibition of sperm motility and viablility is known already. LiCl was declared either autophagy inducer and inhibitor at several reports. 3-MA, an autophagy inhibitor, is commonly studied in cancer for inducing sensitivity and overcoming resistance. In infertility era, a few study can be found and the most relevant one to infertility subject showed that 3-MA has no effect on paternal mitochondria removal at the fertilization.

Study design, size, duration: This study was performed with 40 semen samples collected from normospermic males at İstanbul University, İstanbul Faculty of Medicine, Department of Urology, Section of Andrology between March 2013–January 2014.

Participants/materials, setting, methods: Sperms obtained by the liquefaction of semen of normopsermic males aged between 20 and 35 were divided into four groups as control, LiCl (10 μM), 3-MA (10 μM) and the combination group. Sperms were incubated with the drugs for 1 h at 37°C and then they are evaluated by sperm number and motility (Light microscopy), sperm viability and apoptosis indeces (Flow cytometry), phosphoinositide 3-kinase (PI3-K)/Vps34 protein (Immunohistochemistry, IHC) and ultrastructure (Transmission electron microscopy, TEM).

Main results and the role of chance: Sperm number was decreased by the combination group in comparison to control group and singly applied chemicals (93×10^4; $p > 0.05$). IHC evaluation showed that PI3-K/Vps34 levels were also reduced by the combination group (20%; $p < 0.001$). Viability index was similar to control group and 3-MA group, however it was lower than LiCl group (35.432%; $p > 0.05$). It increased total apoptotic sperm cell rate slightly (54%; $p > 0.05$). TEM evaluation showed that the combination group led to the increase in vacuolisation and apoptotic cell morphology and the decrease in autophagic vacuoles.

Limitations, reasons for caution: Defects were found healthy and normospermic accepted males under TEM evaluation. They are discarded from experiment. Low sample numbers are restrictive to find out exact results for this applications.

Wider implications of the findings: Yet, the effect of LiCl with 3-MA haven't been being tested in male fertility. This study showed that they acted both through the inhibition of autophagy. LiCl with 3-MA will not be new infertility treatment modality for males who suffered from cancer or physcotic disorder or both.

Trial registration number: This study was approved by İstanbul University, İstanbul Faculty of Medicine Clinical Research Ethical Committee at February 19, 2013 and the number is 197.

P-046 Testicular endocrine patterns in young boys operated for cryptorchidism

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Study question: Are hormonal markers of the testicles (Testosterone, inhibin B and Anti-Mullerian Hormon or AMH) in cryptorchid boys are significantly different than in non-cryptorchid boys?

Summary answer: Cryptorchid boys have simultaneous significantly decreased serum testosterone, AMH and inhibin B levels, suggesting a functional defect of both Leydig and Sertoli cells.

What is known already: Cryptorchidism is the most common male congenital malformation, with an increasing incidence within the last decades in industrialised countries. Cryptorchidism is also a major risk factor for infertility and for testis cancer (Toppari, 2001; McGlynn, 2012). Many studies have compared hormonal markers of the testicles (Testosterone, inhibin B and Anti-Mullerian Hormon or AMH) in cryptorchid and non-cryptorchid boys. Nevertheless, the results remain contradictory showing no difference in older studies (Christiansen, 2002; Barthold, 2004; Suomi, 2006), while more recent studies show a significant reduction of these hormones in children with cryptorchidiscryptorchidism (Guibourdenche, 2003; Pierik, 2009; Matuszczak, 2012; Thorup, 2015).

Study design, size, duration: We performed a hospital-based cross-sectional study. During one year, we identified from surgery appointment records, boys with unilateral or bilateral cryptorchidism undergoing orchiopessy (excluding any illness or other urogenital malformations) and boys undergoing dental care, minor osteoarticular (talipes, hallux valgus) or dermal surgery (naevi). Informed parental consent was obtained. Blood samples were taken during the

surgical procedure for biological (cholesterol and triglycerides) and hormone assays (testosterone, inhibin B and AMH).

Participants/materials, setting, methods: Analyses were performed in the Biochemistry department of Toulouse University Hospital.

Cholesterol and triglycerides concentrations were determined enzymatically with an Olympus analyzer (Beckman Coulter, Roissy, France).

Testosterone was assessed through an RIA kit (Cis-Bio International, Gif-sur-Yvette, France).

AMH was assayed by an enzyme-linked immunoassay (ELISA) kit provided by DSL-Beckman Coulter (Webster, TX, USA).

Inhibin B was measured by ELISA with a kit supplied by OBI-DSL (Oxford, UK).

Both AMH and Inhibin B were assayed in duplicate according to manufacturer's protocols.

Main results and the role of chance: Two groups of 27 boys were included, different for age at surgery: 26.6 versus 24.2 months ($p = 0.172$).

All serum levels were lower in cryptorchid than in control boys. Testosterone levels, measured in 10 controls and 10 cases, were 18.10 ng/100 mL ± 4.07 and 11.60 ng/100 mL ± 1.26, respectively ($p < 0.001$). AMH levels were 86.63 ng/mL in cryptorchid boys and 134.56 ng/mL in controls ($p = 0.003$), while inhibin B levels were 96.89 pg/mL and 133.44 pg/mL, respectively ($p = 0.007$).

AMH and inhibin B were markedly lower in the bilateral cryptorchidism subgroup, being 50% lower than in the controls (both $p = 0.003$).

Limitations, reasons for caution: Weakness of the study relates to the absence of INSL3 assay, an additional marker of Leydig cells dysfunction, which could reinforce our results on testosterone secretion in both groups. INSL3 is involved in the trans-abdominal phase of testis descent. Unfortunately, the assay was not available at the time of study.

Wider implications of the findings: Decreased serum testosterone, AMH and inhibin B levels, suggest a functional defect of both Leydig and Sertoli cells. The Sertoli cell defect appeared more pronounced in bilateral cryptorchid boys. It could be interesting to assess the impact on the future fertility of these boys.

Trial registration number: We have obtained the study agreement number 907152 from the French data protection Authority (CNIL).

P-047 Hydrogen molecule treatment enhancesincreases ATP production in human spermatozoa

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Study question: Why does hydrogen molecule treatment enhance ATP in human spermatozoa?

Summary answer: H_2 treatment rescued inhibition of complexes I and III of the mitochondrial electron transport chain. H_2 treatment increases ATP production in spermatozoa.

What is known already: H_2 treatment remarkably improved the rate of forward motility of human spermatozoa, whereas N_2 treatment did not. H_2 treatment also increased the mitochondrial membrane potential of human sperm. Less motile frozen-thawed sperm from patients improved the rate of forward motility after being cultured in a medium containing 75% hydrogen molecules.

Study design, size, duration: Frozen-thawed sperm suspension from 132 normospermic patients were divided into seven groups: control group was H_2 noncontained medium; H_2 treated group was 75% H_2-saturated medium; the other groups were antimycine A (A), rotenone (R) contained each control mediums, or 75% H_2-saturated mediums; and pentoxyphiline (P) group contained control medium.

Participants/materials, setting, methods: Normospermic suspensions were frozen on the collected day. Forty-two sperm suspensions treated A (200 μM), 45 sperm suspensions treated R (200 μM) and 45 sperm suspensions treated P (2 mg/ml). Sperm ATP was measured with an luminometer. Sperm concentration and motility were measured with Makler chamber and Sperm Class Analyser (SCA).

Main results and the role of chance: In terms of sperm ATP amount, 75% of H_2-saturated medium showed the best results compared with those of A (H2 + A vs. C + A: 305.58 ± 204.89 vs. 186.65 ± 111.26, $n = 30$, $P < 0.001$), R (H2 + R vs. C + R: 297.37 ± 157.58 vs. 171.11 ± 77.36, $n = 30$, $P < 0.001$), P (H2 vs. P: 547.01 ± 324.89 vs. 358.84 ± 190.12, $n = 45$, $P < 0.001$), control (H2 vs. control: 437.06 ± 291.47 vs. 306.92 ± 200.01, $n = 105$, $P < 0.001$), respectively (pmol/10^6 sperm: data are shown as mean ± SD). On measuring sperm motility parameters with SCA, C + A treated sperm were significantly lower than H + A treated sperm in VCL, VSL, VAP, ALH, and BCF ($P < 0.05$, $n = 12$). C + R treated sperm were significantly lower than H + R treated sperm in VCL and ALH ($P < 0.05$, $n = 15$).

Limitations, reasons for caution: This is a basic study on a relatively small sample size with limited conditions. The confirmation using larger samples under various conditions may be required. Furthermore, we need to check the safety of H_2 treated sperm use in ART.

Wider implications of the findings: The findings of this study indicate that H_2 treatment increases the intracellular ATP of the oligoasthenoteratozoospermic patients' sperm. Possibly, men with severe sperm dysfunction could select IVF instead of ICSI by using H_2 treatment. It may also be useful in the selection of good quality sperms for ICSI.

Trial registration number: Nothing.

P-048 Patients with Sertoli cell-only syndrome combining with seminiferous tubule hyalinization have higher sperm retrieval rate when micro-TESE was performed

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Study question: Could seminiferous tubule hyalinization predict a different sperm retrieval rate (SRR) when non-obstructive azoospermia (NOA) patients with Sertoli cell-only syndrome (SCO) undergo microdissection testicular sperm extraction (micro-TESE)?

Summary answer: Patients with SCO combining with seminiferous tubule hyalinization had higher SRR comparing with those not combining with seminiferous tubule hyalinization.

What is known already: More intratubular germ cells with active spermatogenesis within seminiferous tubule cause the tubule to appear larger, more opaque and whiter than tubules without sperm production, therefore micro-TESE can find out the small isolated areas of sperm production according to the microscopic findings. SRR has been achieved in up to 22.5–42.9% of patients with SCO, however, testicular volumes and serum level of FSH, LH, inhibin B and testosterone is just indicative of the testicular function as a whole and may not reflect focal areas of spermatogenesis that are found during micro-TESE, and is not reliable in predicting SRR.

Study design, size, duration: This is a retrospective study. From Jan. to Dec.2015, fifty NOA patients underwent micro-TESE and were diagnosed histologically with SCO. They were subclassified into patients with seminiferous tubule hyalinization or without seminiferous tubule hyalinization.

Participants/materials, setting, methods: Micro-TESE was performed at x20 magnification under the operating microscope. Seminiferous tubules were removed and immediately placed within a sperm buffer. Then the tubules were dissected and examined immediately by an embryologist under a phase microscope at x200 magnification. The hyalinization group and non-hyalinization group were compared in age, testicular volumes, FSH, LH, inhibin B, testosterone, SRR and microscopic findings of testicular tubules during operation.

Main results and the role of chance: 34 patients (68%) were diagnosed with SCO combining with seminiferous tubule hyalinization, while 16 patients (32%) without hyalinization. No significant difference was observed in age (35.2 ± 6.7 vs. 33.3 ± 5.1), FSH (32.6 ± 12.8 vs. 27.8 ± 9.3), LH (14.7 ± 6.1 vs. 12.1 ± 5.8), testosterone (3.1 ± 0.9 vs. 3.5 ± 1.2) and inhibin B (15.7 ± 10.4 vs. 28.3 ± 8.3) levels between hyalinization and non-hyalinization group (All P>0.05). However, testicular volume in hyalinization group was lower than non-hyalinization group (4.1 ± 2.3 vs. 5.3 ± 3.7, P<0.05). Spermatozoa were successfully retrieved from 16 patients (32%), SRR of hyalinization group was higher than non-hyalinization group (41.2% vs. 12.5%, P<0.05). 15 patients (30%) showed heterogeneous thickness of seminiferous tubule and 12 of them were found to have testicular sperm (80%). Patients in hyalinization group had more opportunity to show heterogeneous tubule than non-hyalinization group (35.3% vs. 18.8%, P>0.05).

Limitations, reasons for caution: In our study, although patients with SCO combining with seminiferous tubule hyalinization had more opportunity to show heterogeneous testicular tubule than those without hyalinization, more cases should be included because the difference was not significant. And it is unclear that whether the hyalinization precedes or follows the spermatogenic disorder.
Wider implications of the findings: Based on our results, regarding higher SRR, micro-TESE should be recommended to NOA patient with SCO especially for those combining with seminiferous tubule hyalinization. Considering micro-TESE would not provide an advantage if seminiferous tubules show homogeneous thickness, needle biopsy should be performed previously to minimized testicular damage.
Trial registration number: (This is a retrospective study).

P-049 Interleukin-18 and apoptosis of mice Leydig cell during lipopolysaccharide-induced acute inflammatory condition

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Study question: Dose Leydig cell-derived interleukin (IL)-18 induce apoptosis of Leydig cell during lipopolysaccharide (LPS)-induced acute inflammation?
Summary answer: LPS induced Leydig cell apoptosis via death-receptor pathway without increased IL-18.
What is known already: In mouse testes, IL-18 is produced from germ cells, Leydig cells, and resident macrophages and may regulate testicular function via autocrine/paracrine signaling under physiologic conditions. IL-18 is an important cytokine to maintain the homeostasis of testicular cells. However, we reported high dose of IL-18 induced germ cell apoptosis on mice endotoxemia. (Inoue et al., 2015). Bamias et al. (2012) reported the role of IL-18 depends on which cells secrete it. In intestine, endothelial cell-derived IL-18 induced epithelial reconstitution, but lymphocyte-derived IL-18 induced epithelial injury. The amount of IL-18 may be a key determinant of the role of IL-18.
Study design, size, duration: Recombinant interleukin-18 (rIL-18) stimulation: TM3 cells were stimulated with 0.1, 1, 10, and 100 ng/mL rIL-18 and were sampled at 12 hours. LPS stimulation: Mouse Leydig cell line TM3 and macrophage cell line RAW264.7 cells were stimulated with 200 or 1,000 ng/mL LPS and were sampled at 0, 1, 6, 12, 24 and 48 hours. Vehicle: TM3 or RAW264.7 cells were incubated medium only.
Participants/materials, setting, methods: Cells were harvested on 6-well microplate (4×10^5 cells) with DMEM-F12 medium for 48 hours at 37°C, 5% CO_2. Cell viabilities were determined by trypan blue staining. Cleaved caspase-3 and tBID was measured by western blotting to evaluate apoptosis and the pathways. Expressions of Tnf-α, Il-6, Il-18 and Fas mRNA were analyzed using reverse transcriptional real-time PCR. Statistical analysis was performed using a Tukey–Kramer's post hoc test (statistically significant; $p < 0.05$ compared with vehicle).
Main results and the role of chance: High dose (10 and 100 ng/mL) of rIL-18-stimulation induced cleaved caspase-3 expression in TM3 at 12 hours. In LPS-stimulation, expressions of Tnf-α and Il-6 were significantly increased at 1 hours ($p < 0.01$), and decreased to baseline within 6 hours. LPS had no influence on the cell viabilities on TM3 and RAW264.7. Cleaved caspase-3 expressions were increased at 6 hours, and it kept until 48 hours. Expression of Fas was increased at 12 hours ($p < 0.05$), but it of tBID could not be detected. Expression of Il-18 in TM3 were significantly low at 6 and 12 hours ($p < 0.05$, $p < 0.05$, respectively). The expression of Il-18 in RAW264.7 were significantly increased at 6 hours ($p < 0.01$) and kept high level until 24 hours. These results suggested that IL-18 was produced mainly by macrophages during LPS-stimulation. In this study, rIL-18 induced Leydig cell apoptosis. LPS also induced Leydig cell apoptosis via death-receptor pathway, but LPS did not increase IL-18 in Leydig cell. Macrophages increased IL-18 expression by LPS stimulation. We speculated macrophage-derived IL-18 may be a main reason of over-expression of IL-18, which leads Leydig cell apoptosis, during endotoxemia.
Limitations, reasons for caution: The limitation of this study is the cell-cell interactions could not be evaluated. Future studies are planned to investigate the effect of IL-18 on cell-cell interaction by co-culture system.
Wider implications of the findings: Our results suggested that the high dose of IL-18 induced of Leydig cell apoptosis. The high dose of IL-18 may derive from macrophages. Prevent overexpressed IL-18 from macrophages could be a new therapeutic target to prevent orchitis during endotoxemia.
Trial registration number: Not applicable.
Reference
Inoue T., et al., (2015) Reproduction 150:105–114.

P-050 Assessment of sperm motility in oligoasthenospermic men, treated with metabolic and essential nutrients, in a randomized, double blind, placebo study

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Study question: The aim of the study was the assessment of sperm motility, especially progressive motility, after 3 and 6 months therapy of men with oligoasthenozoospermia.
Summary answer: Study showed significant improvement in progressive motility after 6 months therapy with metabolic and essential nutrients in men with initial low progressive motility.
What is known already: Spermatozoa have high-energy requirements for maturation, capacitation and motility. Indeed many factors affecting their motility act through decreasing energy availability mainly by disrupting mitochondrial function. Many nutrients and metabolic compounds affect mitochondrial function. The compounds L-carnitine (L-C) and acetyl-L-carnitine (ALC) play a pivotal role in transport of long-chain fatty acids into the mitochondrial matrix, where they are used to generate energy. Both L-C and ALC also have important roles in the post-gonadal maturation of spermatozoa. Studies have reported either no effect or that treatment with combined high dose L-C and ALC significantly increases sperm concentration and forward motility.
Study design, size, duration: This study was randomized, double blind, placebo controlled and it examined the effect of test formulation, Proxeed Plus, L-C 2g and ALC 1g, as well as vitamins and minerals, in men with oligo- or asthenoteratozoospermia. The protocol was 2 months "wash out" and 6 months therapy (125 patients) or placebo (50 patients) and 2 months follow up (T-2, T0, T+3,T+6). The control group (placebo) 50 patients were administered in a similar way with the placebo.
Participants/materials, setting, methods: Men visiting the Andrology center, aged between 18 and 50 years and with history of difficulty conceiving > 12 months were randomized to receive treatment or placebo in a double blind protocol**.** Subjects with endocrine disorders, autoimmune disease, azoospermia, cystic fibrosis, testicular cancer etc., were excluded. Compliance was assessed at visits. Analysis of ejaculate was done according to WHO 5th guideline, and progressive sperm motility (A+B grade of rapid, progressive) was done manually.
Main results and the role of chance: There was a statistically significant difference with $p = 0.34$, by Kruskal-Wallis test, in the values of progressive sperm motility in three different time period: T0=22.50% (11.50 ± 38.00), T3=22% (11.00 ± 40.00) and T6=28.50% (16.75 ± 40.25).

Then we correlated all 3 groups between each other, with Friedman tests, and analysis showed a statistically significant difference between T0 =22.50% (11.50 ± 38.00) and T6= 28.50% (16.75 ± 40.25) with $p < 0.05$. In placebo group we couldn't find significant improvement, in progressive sperm motility, before 20.14 (10.39 ± 28.11) and after 6 months period (23.44 (14.65 ± 30.16) with $p < 0.082$.
Limitations, reasons for caution: In this randomized, placebo study we used a manual method of evaluating the rapid and progressive movement of the cells, and this can have a some possible impact of assessment of motility. But experienced biologist and technician has done all this analysis
Wider implications of the findings: Results of this randomized study agreeing with with other non-randomized studies with smaller sample size. We underline

the importance of duration of therapy (3 and 6 months) with metabolic and essential nutrients: this means that after 6 months therapy we can expect significantly higher percentage progressive sperm motility.
Trial registration number: Protocol PXP-001-B.

P-051 Outcome of testicular sperm extraction in 52 spinal cord injured-men

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Study question: What are the outcomes of testicular sperm extraction in spinal cord injured-men?
Summary answer: TESE is one of the most useful technique for sperm retrieval in spinal cord injury patient.
What is known already: In men with spinal cord injury (SCI) the ejaculated sperm usually exhibits poor motility and viability. Under these circumstances, IUI with ejaculated sperm in men with SCI commonly is not offered so far. Recently, it is said testicular sperm is optimal for intracytoplasmic injection (ICSI) in men with SCI.
Study design, size, duration: This study is retrospective analysis for spinal cord injury-men who enforced TESE from April, 2008 to December, 2015.
Participants/materials, setting, methods: A total of 52 male patients with ejaculatory disorder due to SCI who underwent TESE for various reasons were included in this study. We investigated the sperm retrieval rates (SRRs) and pregnancy rates (PRs) from the medical records. Data on the age, testicular volume, the hormonal status (lutenizing hormone, follicle stimulating hormone (FSH), testosterone), and the period from SCI injury were also obtained and analyzed to detect any associations with the presence of spermatogenesis.
Main results and the role of chance: SRRs and PRs were 89% and 62%, respectively. A univariate analysis revealed a statistically significant relationship between serum FSH level ($P < 0.001$), serum LH level ($P < 0.005$), the period from SCI injury ($P < 0.005$) and spermatogenesis presence. A logistic regression model, however, revealed a statistically significant relationship only between serum FSH level and spermatogenesis presence ($P < 0.0001$). No association was found between the period from SCI injury and the presence of spermatogenesis.
Limitations, reasons for caution: Study data were obtained retrospectively, which might have affected the quality of the data. Furthermore, the sample size was too small to draw definite conclusions from the results. We need to accumulate additional data from similar cases.
Wider implications of the findings: None.
Trial registration number: None.

P-052 Taste receptors genes variability related to male infertility: expression of gustducin and transducin G proteins α-subunits in human sperm

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Study question: Association of TAS2Rs/TAS1R (taste receptors) and GNAT3 (alpha transducing 3) genetic variants with male infertility and localization of α-gustducin and α-transducin in human spermatozoa before and after *in vitro* capacitation.
Summary answer: Genetic variants in taste related genes and sperm parameters are correlated. Human sperm expresses taste receptors with a different localization in basal versus capacitated sperm.
What is known already: Several studies in mammals have demonstrated the expression of α-gustducin and α-transducin, G proteins coupled with taste receptors, during mouse spermatogenesis and a segmental distribution along the flagellum of mouse, rat, bull, and boar spermatozoa, suggesting a functional role in processing intracellular signals controlling sperm motility. It has been reported that taste sensitivity varies among individuals and that it is associated with polymorphisms in the taste receptor genes. The high frequency of human genetic variations of genes involved in taste perception results in a large variability from super-taster subjects to totally "non-taster" patients, that might be influence sperm functionality too.
Study design, size, duration: For this study, a total of 314 males patients undergoing spermiogram evaluation during infertility diagnosis at the Centre of Couple Sterility, Siena University Hospital (Italy), were enrolled starting from October 2014 to October 2015.
Participants/materials, setting, methods: The patients enrolled were characterized for main sperm parameters, according to WHO (2010) guidelines: concentration, morphology, progressive and total motility, and their genomic DNA was isolated both from buccal swab. All subjects were then genotyped for 24 single nucleotide polymorphisms (SNPs) in TAS2Rs/TAS1R and GNAT3 genes. The genotyping was performed using the KASPar SNP genotyping system. Ejaculated spermatozoa were analyzed by Western blot and immunofluorescence analysis before and after *in vitro* capacitation.
Main results and the role of chance: Association between the SNPs and the main sperm parameters was tested through chi^2 test. We found a statistically significant association between *sperm concentration* and *progressive motility* with TAS1R1 rs12080675 SNP ($p = 0.04$; $p = 0.01$). Also TAS2R49 rs7135018 SNP ($p = 0.01$) was associated with *sperm concentration*. *Progressive and total motility* resulted correlated with both TAS1R1 rs11587438 SNP ($p = 0.001$) and TAS2R14 rs11610105 ($p = 0.014$). A statistically significant association was also between *total motility* and TAS2R44 rs10845293 ($p = 0.015$), TAS2R49 rs7301234 ($p = 0.008$) the TAS2R50 rs1376251 SNPs ($p = 0.004$). Based on immunoblot and immunofluorescence analyses, both G proteins are present in human sperm; α-gustducin was detectable in both the acrosome and the proximal region of the tail, whereas α-transducin signal was confined only to the proximal region of the tail, with a different intensity among individual sperm cells. After capacitation, both G proteins change their localization: this phenomenon may be relevant for the next stages, such as acrosomal reaction, zona pellucida penetration and finally motility iperactivation.
Limitations, reasons for caution: The polymorphism TAS2R49 rs7135018 has a low frequency in the population and therefore a larger study needs to be carried out in order to validate the findings.
Wider implications of the findings: Further studies might contribute in further understanding the genetic components of male infertility and the possible role of these G proteins in sperm physiology.
Trial registration number: None.

P-053 Evaluation and introduction into service of Male Factor Infertility assays, in the provision of Y-chromosomal micro-deletion screening for the West of Scotland

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Study question: To evaluate the suitability of two newly developed assays for the detection and determination of the extent of Y-chromosomal micro-deletions in routine diagnostic practice
Summary answer: We document our experience of Elucigene Diagnostics Male Factor Infertility kits (MFI) that are suitable for diagnostic service provision and compliant with EMQN guidelines
What is known already: Chromosome micro-deletions of the Azoospermia factor regions (AZF) on the Y chromosome have long been implicated in male infertility. There are several commercial Y-chromosomal micro-deletion analysis kits available, however recent EMQN best practice guidelines recommend that the extent of any deletion detected is determined (using extension analysis) for the fullest clinical representation and utility. Elucigene Diagnostics have produced a workflow (MFI) to detect AZF deletions and then ascertain their extent. In addition the MFI kit can indicate of the presence of a sex chromosome aneuploidy, itself a cause of male infertility.
Study design, size, duration: Retrospective testing of Y micro-deletion positive samples from our diagnostic service male infertility cohort and parallel analysis of prospective samples referred during (Oct-Dec 2015). Further data will be collected throughout 2016 as the assays are phased into service delivery.

Participants/materials, setting, methods: Blood samples from male patients referred from local (Greater Glasgow and Clyde NHS region, Scotland, UK) Assisted Conception Units, in male infertility cases for routine genetic analysis including karyotype analysis.
Main results and the role of chance: Our study comprises retrospective and prospective testing AZF deletion cases (known positives or new referrals) and then ongoing service delivery using the Elucigene Diagnostics Male Factor Infertility assays. Our testing remit includes the use of both Male Factor Infertility and Male Factor Infertility-Y-Plus to fully resolve any deletions detected. In total we analysed 34 retrospective cases and 35 new referrals with the MFI kit.

Secondary analysis was performed using Elucigene Diagnostics MFI-Y-Plus assay to assess the extent of those deletions found with the MFI assay. We analysed a total of 8 cases with deletions which reflected a range of AZFa, AZFb+partial AZFc and AZFc deletions.

Our results showed complete concordance with previous or parallel analysis using an alternative Y-chromosome micro-deletion kit and additionally we were able to define the extents of those deletions. This included the ability to discriminate between two AZFb+partial AZFc deletion cases which were previously typed as identical, but differed with the presence or absence of marker sY105.

Implementation of these kits proved to be simple, with single tube set up for both assays and clear and intuitive analysis using Soft Genetics Genemarker™. Adoption of this workflow has facilitated improvements to our Infertility service.
Limitations, reasons for caution: Due to the nature of our referrals no new deletions or sex chromosome aneuploidy were detected during the prospective validation period (Oct-Dec 2015). While the markers present are sufficient to indicate the presence of a sex chromosome aneuploidy, we recommend additional analysis by sexing QF-PCR for confirmation.
Wider implications of the findings: A solution to the difficulties of introducing extension analysis for the Molecular Diagnostic community of accredited service laboratories in the UK and beyond.
Trial registration number: Not applicable.

P-054 Mitochondrial DNA copy number in spermatozoa as a predictor of successful IVF

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Study question: Is there an association between mitochondrial DNA (mtDNA) copy number in spermatozoa and IVF outcome?
Summary answer: No significant correlation was found in the studied IVF group between the number of mtDNA copies and fertilization rate or pregnancy outcome.
What is known already: Sperm mitochondria provide energy via adenosine triphosphate (ATP) production and affect apoptosis (programmed cell death). Abnormal mitochondria may therefore effect sperm motility and are associated with reduced spermatozoa production. Several studies reported mutations in mtDNA associated with asthenozoospermia or a negative impact on male fertility. Deletions in mtDNA and decreased sperm motility have also been described. Studies evaluating the relationship between abnormal semen parameters and mtDN copy numbers found that an increased mtDNA copy number could result in parameters such as a higher percentage of non-progressive motile spermatozoa and thus influence the fertilizing capacity of spermatozoa.
Study design, size, duration: Prospective observational cohort study. Semen samples were collected from 104 men of couples undergoing IVF treatment in the IVF centre of a university medical centre.
Participants/materials, setting, methods: Participants of the study were males from couples undergoing IVF treatment in a university hospital. The threshold for inclusion was ejaculates with a minimal post-wash sperm count of $>10^6$ spermatozoa. Semen analyses were performed based on the WHO (1992/1999) guidelines. The method for mtDNA measurement used was based on the commercially available Retina Mitox DNA test (Primagen – Amsterdam, the Netherlands) consisting of DNA extraction, DNA amplification and mtDNA measurement.
Main results and the role of chance: To examine the correlation between the number of mtDNA copies and the fertilization rate in the IVF group, the Spearman's Rho Correlation coefficient for non-paramentric data was used. No significant correlation was found, $r = 0.068$, $p = 0.494$. The mean (SD) fertilization of oocytes in the study group was 66.7% (28.3). The correlation between mtDNA copy number and pregnancy outcome was also non-significant, $r = -0.45$, $p = 0.654$. The overall pregnancy rate was 32.4%.

Data concerning fertilization were non-dichotomous. A cut-off value was visually determined for fertilization rates, in order to use the dichotomous ROC curve. The cut-off value was 21%. The "area under the curve" (AUC) was used as a measurement of accuracy of the test, which was 0.611. The small AUC indicates that the number of mtDNA copies has no predictive or prognostic value for fertilization rates in IVF treatment.

The ROC curve was also used for studying the predictive value of mtDNA copy number for the outcome of pregnancy, but yielded similar results, indicating that mtDNA copy number in sperm cells does not have a predictive value for pregnancy outcome in assisted reproductive treatment.
Limitations, reasons for caution: A limitation is the fact that one needs at least 10^6 total progressive motile spermcells post-wash to adequately run the Retina Mitox DNA assay. Therefore males with less than 10^6 post-wash spermatozoa were excluded from the analyses.
Wider implications of the findings: Based on this study mitochondrial DNA in spermatozoa has no prognostic value in assisted reproductive therapies regarding fertilization rate or pregnancy outcome.
Trial registration number: No clinical trial.

P-055 Study of the protection of zinc and alpha-tocopherol on human sperm parameters, lipid peroxidation, apoptosis and DNA fragmentation with Mobile Phone Radiation treated *in vitro*

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Study question: What is the role of zinc and alpha-tocopherol to protect human sperm irradiated with mobile phone?
Summary answer: its seems zinc and alpha-tocopherol decrease the harmful effect of mobile phone exposure on human sperm.
What is known already: Cell phones have become an essential part of daily life but, the health risks related with their usage are often overlooked. However, possible consequences of the cellular phone usage on human sperm parameters have not been investigated adequately. This study was performed to evaluate the possible protective effect of zinc and alpha-tocopherol, against sperms exposed to the cell phone radiation.
Study design, size, duration: Semen samples were obtained from 18 fertile males presenting to the infertility clinic between 25–41 age years during 1 Feb 2013 t0 1Feb 2014. Following liquefaction, sperm parameter was performed based on WHO standard criteria (2010), and then each sample was divided equally into 5 parts: control (sample not exposed), exposed (sample exposed to cell phone), exposed+15µM zinc, exposed+5µM alpha-tocopherol, and exposed+15µM zinc+5µM alpha-tocopherol groups. Irradiation was done by cell phone for 10 minutes continuously.
Participants/materials, setting, methods: In all groups, sperm analysis was performed for their viability, morphology and motility. Morphology test was conducted using giemsa dye, viability test with trypan blue staining protocol and DNA fragmentation with halo test. Apoptosis detection was carried out by means of TUNEL kit, lipid peroxidation via MDA kit and total antioxidant capacity with TAC kit. Data were analyzed by one-way ANOVA followed by Tukey's test using SPSS version 16 software.
Main results and the role of chance: Our result indicated that exposure group showed a significant decrease in the rapid progressive, slow progressive sperm movement and viability compare to control groups. It also increases the no – motility category of sperm movement and abnormal sperm significantly ($p < 0.05$). In comparison with the fresh and exposed groups, there was a significant decrease in the total antioxidant capacity but increased the lipid peroxidation, DNA fragmentation and apoptosis ($p < 0.05$).

Also alpha- tocopherol (5 mM) and zinc (15 µM) increases rapid progressive, slow progressive and viability of sperm and decreased abnormal sperm in mobile phone exposed group significantly. Moreover, zinc and

alpha-tocopherol only and combined together, induced significant increases the total antioxidant capacity and decreased lipid peroxidation, DNA fragmentation and apoptotic sperm in mobile phone exposed group ($p < 0.05$). Furthermore, there was not a significant difference in morphology between five groups.

Limitations, reasons for caution: This is an in-vitro study because of we used just human fertile men just presenting to our infertility center and the number of case low during study.

Wider implications of the findings: We concluded Radiofrequency electromagnetic waves emitted from cell phones may lead significant decrease sperm parameter**.** The addition of zinc and alpha- tocopherol only and combined together in the semen could be useful to prevent human semen parameters after mobile phone radiation.

Trial registration number: The results of this study indicated that master of science thesis in guilan university of medical sciences with No: 100.

P-056 Is there an association between the different HOST-induced tail swelling patterns and sperm quality?

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Study question: We aimed to investigate whether the degree of sperm tail swelling observed during hypo-osmotic swelling test (HOST) may predict sperm quality in infertile men.

Summary answer: Our results show that the pattern of sperm tail swelling seems to be strongly linked to sperm functional integrity.

What is known already: Based on the sperm reaction (swelling) under hypo-osmotic conditions, HOST was developed to evaluate sperm membrane integrity. The usefulness of this test to identify viable immotile sperm in testicular or ejaculated preparations during intracytoplasmic sperm injection (ICSI) is widely approved. However, little is known about the significance of the different patterns of sperm tail swelling under hypo osmotic stress.

Study design, size, duration: The study was performed on semen samples collected from 37 men attending our center for couple infertility investigation.

Participants/materials, setting, methods: Semen analyses and HOST were performed according to the World Health Organization (WHO) guidelines. Sperm chromatin status was evaluated by a modified version of the Diff-Quik staining assay and expressed by the percentage of abnormal dark nuclear staining sperm (DQ-D).

Main results and the role of chance: The mean HOST value for the study group was 66.5 ± 10%, and was positively correlated with sperm motility ($r = +0.34$; $p = 0.03$) and vitality ($r = +0.34$; $p = 0.04$). There was a negative association between the mean HOST value and the percentage of DQ-D sperm ($r = -0.36$; $p = 0.03$). The mean percentage of HOST-induced tail-swelling grade "b" was significantly associated with higher sperm motility, vitality, concentration, and improved sperm morphology. Grade "d" was positively correlated with sperm motility and morphology, and grade "f" with sperm concentration and morphology.

Limitations, reasons for caution: The distribution of HOST categories with relevance to semen quality subgroups need to be elucidated.

Wider implications of the findings: HOST is a useful tool for rapid, low-cost semen quality evaluation. According to our results, HOST grades "b", "d" and "e" were most associated with better sperm parameters, suggesting their preferential use during routine sperm selection for ICSI.

Trial registration number: No trial registration number.

P-057 Cigarette smoking and semen quality: a new meta-analysis examining the effect of the 2010 world health organization laboratory methods for the examination of human semen

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Study question: To assess the impact of smoking on semen parameters according to the updated World Health Organization (WHO) methods for laboratory evaluation of human semen.

Summary answer: Cigarette smoking negatively affect sperm parameters. Sperm count and motility remained similar when WHO 2010 and earlier criteria were used but equivocal for sperm morphology.

What is known already: Approximately 37% of men of reproductive age smoke cigarettes, with Europe having the highest tobacco use among all the WHO regions. Smoking cigarettes has been associated with a deterioration of sperm quality, including motility, concentration and morphology, which are the parameters most frequently used in clinical settings to assess fertility. However, the evidence is not unequivocal, and some studies have found no effect on sperm quality. Given the high prevalence of smoking and the new WHO laboratory criteria for the examination of human semen, the role of cigarette exposure on semen parameters needs a new look.

Study design, size, duration: We conducted a systematic search using MEDLINE/Pubmed, SJU discover and Google Scholar to identify all relevant studies published from 2010 to 2015 (August) after release of the latest WHO methods for laboratory evaluation of human semen. Participants were from fertility/urological clinics and andrology laboratories. The outcome measures were semen volume, sperm concentration, motility and morphology which are the parameters commonly used in clinical settings to assess fertility.

Participants/materials, setting, methods: Participants were males aged 13 years and older regardless of population size and origin. Each semen parameter was evaluated separately and independently. Infertile smokers were compared to matched smokers from the general population. Subgroup analyzes included the comparison of infertile smokers and infertile non-smokers, WHO criteria for laboratory examination of human semen 2010 edition versus previous editions, and impact assessment of the number/amount of cigarettes consumed per day on semen parameters.

Main results and the role of chance: We used twenty studies in the meta-analysis, involving 5,865 subjects. Exposure to cigarette smoking was associated with reduced sperm count (mean difference -9.72×106/mL, [95% CI −13.32, −6.12], $P < 0.001$), motility (mean difference −3.48% [95% CI −5.53, −1.44], $P < 0.001$) and morphology (mean difference −1.37%, [95% CI −2.63, −0.11], $P = 0.03$), but not with semen volume (mean difference −0.15 mL, [95% CI −0.35, 0.05], $P = 0.14$). Subgroup analyses indicated that effect size was higher in infertile men than the general population and in moderate (10–20 cigarettes a day) and heavy smokers (>20 cigarettes a day) than mild (<10 cigarettes a day) smokers. The recent changes in the WHO methods (5th edition) for laboratory evaluation of human semen did not seem to influence the observed negative effect of an association between smoking and sperm count and motility, but results on semen volume and morphology were equivocal.

Limitations, reasons for caution: The meta-analysis did not include any randomized trials since a randomized trial to assess the impact of smoking is not practical. We conducted a thorough bias evaluation of the included studies.

Wider implications of the findings: Pooled results from studies evaluated suggest that cigarette smoking negatively affects sperm quality. The full clinical implications of the WHO 2010 criteria on the association between cigarette smoking and human semen quality deserves further investigation.

Trial registration number: As a meta-analysis the study did not require registration.

P-058 Clinical experience with personalized treatment of male infertility by FSH based on FSH receptor gene polymorphisms

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Study question: Can the low-dose FSH treatment of oligoastheno (terato) zoospermia men based on FSHR gene polymorphisms improve semen analysis parameters and pregnancy rate?

Summary answer: The low-dose administration of FSH in oligoastheno (terato) zoospermic men has a positive impact on parameters of semen analysis and pregnancy rate.

What is known already: Follicle-stimulating hormone (FSH) stimulates proliferation of Sertoli cells and it is an essential hormone for induction and maintenance of normal spermatogenesis. FSH acts through binding to a specific receptor (FSHR) encoded by FSHR gene. The two most common polymorphisms in the coding region occur at nucleotide position 919 and 2039 in exon 10, which cause amino acid exchange from threonine (Thr) to alanine (Ala) at codon 307 and from asparagine (Asn) to serine (Ser) at codon 680. These polymorphisms can be risk factors for sperm production. The part of oligoastheno(terato)zoospermic men can benefit from FSH treatment based on their genotype.
Study design, size, duration: Prospective evaluation of the response of low-dose FSH treatment in terms of sperm production and pregnancy rate on the basis of Ala307Thr-Asn680Ser polymorphisms in the FSHR gene in OAT men with normal hormonal profile in 2012–2015 years with the goal to overcome the side-effects and to bring to the patients a low-cost treatment of male infertility.
Participants/materials, setting, methods: We examined 72 Slovak men with presented severe form oligoastheno(teratooospermia with unsuccessful previous IVF cycles. All of them had normal hormone profile, normal karyotype, no cystic fibrosis gene mutation and no Y-chromosome microdeletion. The patients after chemo-/radiotherapy were excluded. We tested FSHR gene exon 10 polymorfisms (rs6165, c.919A>G and rs6166, c.2039A>G) by DNA sequencing. Men with at least one Asn680Ser allele were treated by 75 IU FSH, administrated 3-times one injection weekly.
Main results and the role of chance: DNA analysis revealed 65 men (90.3%) with at least one Asn680Ser allele presented. All of them were treated by low-dose FSH. Semen analysis was done before and 3 months after therapy. The improvement of its parameters was observed after treatment (sperm concentration 3.8 mil/ml vs. 10.8 mil/ml, motility 2.0 mil/ml vs. 5.2 mil/ml, 3% vs. 8% of normal sperm morphology). 16 couples (24.6%) were pregnant 4–8 months after treatment, 10 of them by IVF methods and 5 couples had spontanneous pregnancy. Seven men with standard haplotype (presence of two normal alleles for both polymorphisms in exon 10) were additionaly tested for polymorphisms in the promotor of FSHR gene (rs1394205, −29G>A) and in the promotor of beta-subunit FSH gene (FSHB: rs10835638, −211G>T) by DNA sequencing. This additional examination revealed that 3 of them (3/7, 42.8%) could have profit based on FSH treatment and the chance to have child.
Limitations, reasons for caution: The low-dose and low-cost FSH therapy can be appropriate in selected oligozoospermic men with normal FSH level. The occurence of a higher number of the men with two Ser680 alleles could be affected by method of selection. Moreover, a part of patients can benefit after additional evaluation of FSHR/FSHB promotor polymorphisms.
Wider implications of the findings: We choosed a low-dose FSH administration of normogonadotrophic men with decreased semen parameters to overcome possible side-effect of treatment. According to our preliminary experience and publicated data FSHB and FSHR promotor polymorphisms should be taken into account, including the severity of impairment of spermatogenesis and the genotype of the patients.
Trial registration number: Not applicable.

P-059 The influence of hyperbaric oxygenation (HBO) and antioxidant therapy (AT) on spermatogenesis in course of preparation for assisted reproduction technique (ART)

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Study question: Our study was focused on the comparison of solo AT, HBO effects and combine therapy (CT) in the correction of severe forms of pathospermia.
Summary answer: Merged effect of CT is supreme to the effect of solo AT and HBO on spermatogenesis in the course of preparation in IVF/ICSI cycles.
What is known already: Dramatic idiopathic spermatogenesis changes are known to decrease ART success rate. Oxygen deficiency, piling up of byproducts of anaerobic metabolism can cause disturbances in tissue metabolism, mitochondrial deficiency, imbalance of spermatogenesis of hormonal regulation, and, as a consequence severe forms of pathospermia.

Several studies have shown that empirical AT may improve sperm quality, ART outcome and increase pregnancy rates. There are few studies showing positive effects of hyperbaric oxygenation (HBO) on spermatogenesis.
Study design, size, duration: Data was collected from May 2013 to July 2015. Only the couples with idiopathic male factor of infertility were considered. We analyzed 56 ART failure (IVF/ICSI) cases. Patients were randomly divided into 3 groups. First 16 men – AT (E vitamin, L-carnitin, selenium) for 8 weeks, second 19 men - HBO (12 consecutive sessions, 1,6 ATM for 50 min), third 21 men - HBO and AT (CT). After the therapy the couples underwent subsequent IVF-ICSI.
Participants/materials, setting, methods: The mean men age was 31.6 ± 5.2 years. All of patients had dramatic idiopathic changes in sperm (oligoasthenoteratozoospermia). None of them had any urological and genetic abnormalities. Semen analysis (WHO 2010), DNA fragmentation index (DFI) (Halosperm©), semen oxidative stress reaction (ROS) were performed before and after the treatment. The fertilization rate, blastocyst formation rate, biochemical and clinical pregnancy rates per IVF/ICSI cycle were measured.
Main results and the role of chance: There was no significance difference in parameters in groups before the therapy. The results after the treatment: 1 group – mean sperm concentration increased by 13.9%, sperm motility (a+b) increased by 20.7%,% normal morphology increased by 16.7%, sperm vitality - by 18%, DFI decreased by 6.6% ($p < 0.05$), ROS decreased by 24.1%.

2 group - mean concentration increased by 14.6%, motility increased by 38.6% ($p < 0.05$),% normal morphology increased by 55.6% ($p< 0.05$), sperm vitality - by 24.5% ($p< 0.05$), DFI decreased by 1.6% ($p< 0.05$), ROS increased by 168% ($p< 0.001$).

3 group - mean concentration increased by 20.3% ($p < 0.05$), sperm motility – by 83.3% ($p < 0.001$), percentage of normal sperm increased by 100% ($p < 0.001$), sperm vitality increased by 34.8% ($p< 0.001$), DFI decreased by 5.6% ($p < 0.05$), ROS increased by 142.9% ($p < 0.001$). There was a significant negative correlation between DFI and sperm motility in all groups ($p < 0.05$).

For the couples in 3 groups who underwent ICSI blastocyst formation rate improved to 45% ($p = 0.05$), 46% ($p < 0.05$) 52% ($p < 0.001$) in groups 1, 2 & 3 respectively. Biochemical pregnancy rates were 62.5% [10/16], 63.2% [12/19], 76.1% [16/21] in groups 1, 2, 3 respectively. Clinical pregnancy rates were 43.7% [7/16], 47.3% [9/19], 57.1% [12/21] in groups 1, 2, 3 respectively.
Limitations, reasons for caution: There were no registered side effects of the proposed therapy. Further studies with larger population are needed.
Wider implications of the findings: Merged effect CT is supreme to the effect of solo AT and HBO on semen quality. Despite rising ROS we did not marked increasing sperm DFI. CT significantly improves IVF/ICSI outcome. HBO is proven to be safe and might be used for pathospermia correction in course of preparation for ART.
Trial registration number: N/A.

P-060 Outcome of ICSI with testicular spermatozoa obtained through microscopically assisted testicular sperm extraction in relation to the ovarian response

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Study question: To determine whether the outcome of ICSI with testicular spermatozoa obtained microscopically assisted testicular sperm extraction (m-TESE) is dependent on the number of MII oocytes.
Summary answer: The number of the mature oocytes is an important prognostic factor in ICSI with testicular spermatozoa obtained from azoospermic men.
What is known already: Retrieval of healthy oocytes is a key component of assisted reproductive treatment for infertile couples. Intracytoplasmic sperm injection (ICSI) has been commonly used for couples with male infertility since its first successful introduction in 1992 and reliable pregnancy rates were achieved by using testicular spermatozoa from patient with azoospermia.

Study design, size, duration: A retrospective cohort of women 40 years old who underwent ICSI treatment with testicular spermatozoa were included from 2006 to 2013.
Participants/materials, setting, methods: Women were enrolled only for one cycle. ICSI was performed with motile testicular spermatozoa obtained from 89 men with obstructive azoospermia and 251 men with nonobstructive azoospermia. GnRH antagonist protocol was used for ovulation induction. Simple linear regression was carried out between the number of MII oocytes and the live birth rate. Receiver operator characteristic (ROC) curves were formed to detect a cut-off number of MII oocytes below which live birth rate was significantly decreased.
Main results and the role of chance: The live birth rate was significantly higher in ICSI-mTESE cycles with ≥7 MII oocytes than that with <7 oocytes (30.6% vs. 14.3%, respectively, $r = 0.12$, $p = 0.02$). LBRs were the lowest in cycles with one or two MII oocytes available (9% and 9%, respectively), but these rates were not statistically different than cycles with 3,4,5 and 6 MII oocytes (14.8%, 16.6%, 17.8%, 23%, respectively, $p>0.05$). Embryo transfer was not achieved in 37 cycles with <7 oocytes (37/167, 22.1%) and 18 cycles with ≥7 oocytes (18/173, 10.4%) because of the absence embryos available following ICSI ($p < 0.01$).
Limitations, reasons for caution: The limitations of the present study were the retrospective design and relatively small number of ICSI cycles with testicular spermatozoa.
Wider implications of the findings: Our current findings imply that obtaining low numbers of oocytes is associated with impaired pregnancy rate in ICSI with m-tese cycles.
Trial registration number: Instutional Ethics committe number: 2016-012.

P-061 stress preconditioning of human sperm for cryopreservation: a new strategy to improve frozen-thawed sperm quality

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Study question: pre-freezing sublethal stress Can improve sperm function after freezing. What is the optimum level of Nitric Oxide for preconditioning of sperm before cryopreservation?
Summary answer: Motility, viability and the rate of apoptotic sperm were significantly improved using 0.01 µM Nitric Oxide after freezing.
What is known already: In recent years, controllable sublethal stress due to increasing of general resistance to further stresses have been applied for oocyte, embryo and sperm. Regard the various physiological effects of Nitric oxide, it can be a novel stressor for human sperm cryopreservation. low concentrations of NO can induce biosynthesis kinetics of stress-related proteins (Heat Shock Protein, Intracellular antioxidants including glutathione). Stress-induced proteins reduce activation of the apoptotic cascade, thereby protect cells against oxidative stress. This response is regulated at transcriptional, translational or posttranslational levels.
Study design, size, duration: Semen samples were collected from normozoospermic men ($n = 42$) for assessment of oxidative stress markers (ROS-TAC score). Then, twenty six samples which had not the oxidative stress were selected for experiment.
After processing sperm with PureSperm gradient, each sample was divided into 7aliquots according to the following groups: fresh, groups exposed to 0, 0.01, 0.1, 1, 10 and 100 µM Sodium Nitroprusside (NO donor) for 1 h at 37°C (5% CO2) before cryopreservation.
Participants/materials, setting, methods: The optimum concentration of NO was determined by evaluation of motility and velocity parameters using Computer assisted semen analysis (CASA),apoptosis status (Active caspase detection by flow cytometry), DNA fragmentation (SCSA by flow cytometry) after freezing-thawing.
Main results and the role of chance: Data were analyzed using SPSS and Statistical differences among various group means were determined by ANOVA and Tuckey's post hoc test. the values of $P < 0.05$ were considered to be statistically Significant.

In comparison with the fresh spermatozoa, there was a significant decrease in the viability, motility, velocity parameters and increase in Caspase+/PI- and DNA Fragmentation in the cryopreserved spermatozoa ($P < 0.001$).

Moreover, 0.01 µM NO produced the higher significant percentage of total and progressive motility (62.23 and 44.935% vs 49.43, 34.02% respectively) in compared to group without any treatment (N0-0).

Furthermore, significantly improves average path velocity (VAP), velocity straight linear velocity (VSL) (50.73 and 37.3 vs 64.04 and 48.1 µm/s respectively).

The percentage of caspase 3 activity significantly reduced in the NO-0.01 when compared to the other groups ($p < 0.01$). Also, NO-10 and NO-100 produced the higher percentage of dead spermatozoa (44.8 and 52.9% respectively) compared to the other groups. For DNA fragmentation there was no significant different in NO-0.01 group compared to group without any treatment.(N0-0).
Limitations, reasons for caution: proteins associated with stress and information about the molecular background of elevated stress tolerance induced by NO could be assessed in this experiment. But due to insufficient sperm cells, we couldn't do Proteomic analysis at the same time.
Wider implications of the findings: This approach may improve the efficacy of most of the assisted reproductive techniques such as fertilizing ability, and developmental competence of embryos and gametes.

This approach may result in considerable improvement from a wide range of procedures (*in vitro* fertilization, cryopreservation, etc.) in assisted reproductive techniques.
Trial registration number: N/A.

P-062 How long should we keep ejaculatory abstinence (EA) before intrauterine insemination (IUI)?

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Study question: Is ejaculatory abstinence before IUI essential factor for pregnancy?
Summary answer: The period of ejaculatory abstinence before IUI is not related with pregnancy rates.
What is known already: The total number of motile sperm inseminated has been cited as the most predictive index of conception in IUI cycles. Intercourse on the day of hCG administration might cause concern about a lower recovery of total motile sperm. However, optimal period of ejaculatory abstinence before IUI is not yet established.
Study design, size, duration: Retrospective analysis of 804 IUI cycles performed between April and December in 2015. The period of ejaculatory abstinence before IUI, semen analysis parameters of the ejaculate (pre-wash) and the insemination specimen (post-wash) for IUI, and pregnancy rate following ovulation induction and IUI were recorded.
Participants/materials, setting, methods: The whole ejaculate was prepared for insemination using a technique of density gradient separation with centrifugation. Ejaculatory period before IUI was divided into 3groups (0–2 days, 3–5days, >5 days).
Main results and the role of chance: In each group, age, semen volume, sperm motility (pre-wash) and liquefaction time did not show significant differences. Although the longer EA period, the more total motile sperm count (pre-wash) was observed (88.2, 137.2, 194.7(*10^6/ml), $p < 0.05$), but there were no significant differences in post-wash total motile sperm count (8.3, 10.9, 11.7 (*10^6/ml), $p > 0.05$). Total pregnancy rate was 19.4% (144/804). There were no significant differences among three groups in the pregnancy rate (22.5% (14/80), 18.7% (53/310), 19.3% (77/414); $p > 0.05$).
Limitations, reasons for caution: To identify the period of EA, we asked for questionnaire survey to patients. So, there could be recall bias. Prospective randomized studies with larger sample size will be required to confirm our conclusions.
Wider implications of the findings: There were few studies about relation between pregnancy rate and period of EA before IUI. Our result shows that period

of EA before IUI does not affect pregnancy rate. Therefore, we can advise that abstinence before IUI and the interval of intercourse seldom affect pregnancy outcome.
Trial registration number: None.

P-063 Effect of the chemotherapy cyclophosphamide and busulfan on the levels of pre-meiotic markers and testicular growth factors of sexually immature and mature mice

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Study question: What is the effect of the chemotherapy cyclophosphamide and busulfan on the levels of pre-meiotic markers and testicular growth factors of immature and mature mice?
Summary answer: They decrease the levels of the examined pre-meiotic markers and in parallel increased the levels of the testicular growth factors.
What is known already: Spermatogonial stem cells (SSCs) proliferate and differentiate to sperm is under the regulation of Sertoli cells and other testicular somatic cells. These effects are performed through the production of different growth factors such as glial cell-derived neurotrophic factor (GDNF), stem cell factor (SCF), leukemia inhibitory factor (LIF) (by Sertoli and peritubular cells) and colony stimulating factor-1 (CSF-1) (by Leydig cells). Cyclophosphamide (CP) and busulfan (Bu) are chemotherapy that affect proliferating cells, including spermatogonial cells in the testis, and may cause azoospermia. The effect of CP and Bu on the activity of testicular somatic of immature mice was not study yet.
Study design, size, duration: Mice were injected intraperitoneal (i.p) with 100 mg/kg CP (for immature) or 200 mg/kg (mature) and saline for the CT group, once a week during 3 weeks. The Bu group was injected (i.p) with a single dose of 45 mg/kg Bu or DMASO (1:1 water) for the CT group. Mice were sacrificed every week after the last injection for 5–12 weeks.
Participants/materials, setting, methods: Seven days-old and 8-weeks-old ICR mice were divided into two groups: CP or Bu groups and control group (CT); n = 9–12mice/time point. Testes weights were performed and later on homogenized for protein evaluation and RNA extraction. Homogenates were examined for protein levels of the above factors by specific ELISA and for RNA expression by qPCR analysis.
Main results and the role of chance: Our results show an increase in the production (at the level of protein and RNA) of all the examined growth factors GDNF, SCF (originate from Sertoli cells) and CSF-1 (originate from Leydig cells) mainly within the first three weeks after the last injection in the CP or Bu groups compared to the CT groups. Thereafter, their levels were similar between CP and CT group or Bu and CT group. The increased levels of those growth factors were in parallel to decrease in the expression levels of the examined pre-meiotic markers (vasa, sall4 and GFRa). The effect of CP and Bu on the production of growth factors was similar in both immature and mature mice.
Limitations, reasons for caution: The study was performed in mouse model and the correlation with human should be considered.
Wider implications of the findings: This is the first study that shows effect of CP/Bu on testicular somatic cell activity in immature and mature mice and the negative correlation with the expression of pre-meiotic markers. Our results emphasize the crucial cross talk between somatic cells and spermatogonial cells during gonadal cytotoxicity to cure spermatogenesis.
Trial registration number: NA.

P-064 BrdUTP/anti-BrdUTP TUNEL labeling system fails to detect DNA fragmentation in highly condensed chromatin of dead/apoptotic spermatozoa

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Study question: Is antibody-based TUNEL (Terminal deoxynucleotidyl transferase dUTP Nick End Labeling) adequate to analyse spermatozoa DNA fragmentation (SDF) in the assessment of male infertility?
Summary answer: The labeling system BrdUTP/anti-BrdUTP greatly underestimates the value of DNA fragmentation in dead and apoptotic spermatozoa.
What is known already: Although many studies reported adverse effects of SDF on the outcome of assisted reproduction technology, there are still considerable discrepancies between SDF values and the correlation between SDF thresholds and male infertility. These uncertainties are one of the reasons why several Societies (ASRM, ESHRE) still do not recommend SDF to be introduced into clinical practice. The TUNEL assay is one of the most commonly used methods for SDF quantification. The labeling system BrdUTP/anti-BrdUTP is described as giving rise to brighter signals in flow cytometry because of a more readily incorporation of BrdUTP into the DNA of apoptotic cells.
Study design, size, duration: This study was part of the validation of the TUNEL assay coupled to flow cytometry analysis in the Clinic of Gynecological Endocrinology and Reproductive Medicine, University Hospital, University of Basel, Switzerland and involved the TUNEL analysis of 20 semen samples of fertile and infertile men (WHO 2010 reference values) attending the Clinic of Gynecological Endocrinology and Reproductive Medicine between July 2015 to January 2016.
Participants/materials, setting, methods: In this work, TUNEL assays with different labeling systems were tested. Br-dUTP/anti-BrdUTP system was analyzed with the ApoBrdU DNA Fragmentation Assay Kit (BioVision Inc.) and the fluorescein- labeled nucleotide system with In Situ Cell Death Detection Kit, Fluorescein (Roche). The influence of chromatin condensation was proved by decondensation of spermatozoa DNA followed by TUNEL. The efficiency of labeling in different populations of spermatozoa was obtained with sorting and TUNEL.
Main results and the role of chance: We demonstrated that BrdUTP/anti-BrdUTP labeling system largely underestimates DNA damage in semen analysis. Compared to the Roche kit, which directly labels the nucleotide, BrdUTP/anti-BrdUTP stained as low as 3% of the spermatozoa stained with the Roche kit. We found that the differences in efficiency of the labeling depend on the condition of the spermatozoa: BrdUTP/anti-BrdUTP staining of dead/apoptotic spermatozoa is very low but staining of living spermatozoa is identical in both methods. We conclude that the difference in the staining efficiency is due to the sterical hindrance effect of the antibody against BrdUTP. The antibody is too large to access the highly condensed form of dead/apoptotic spermatozoa chromatin. After decondensation of dead spermatozoa DNA, BrdUTP/anti-BrdUTP proved to be able to fully detect the DNA breaks. The use of labeled antibodies revealed to be inadequate for detection of SDF. Nevertheless, in both systems, and depending on the semen sample, there is a considerable percentage of unstained dead spermatozoa, confirming the results of Mitchell et al. describing that TUNEL results underestimate SDF due to chromatin condensation. The relationship of TUNEL staining efficiency on spermatozoa/chromatin state was, to our best knowledge, never reported so far.
Limitations, reasons for caution: Although there are several companies selling TUNEL kits with the system BrdUTP/anti-BrdUTP, only BioVision kit was tested. We assumed that the other kits with the same labeling system will present identical limitations in spermatozoa staining.
Wider implications of the findings: This work has shed some light on the problem of TUNEL discrepancies between different laboratories contributing to the standardization of protocols. Our findings on TUNEL efficiencies dependence on the spermatozoa state emphasize the need to carefully interpret TUNEL results comparison between semen samples and processed samples (>90% spermatozoa alive).
Trial registration number: NA.

P-065 The effect of varicocelectomy on semen parameters and pregnancy rate in infertile male with clinical varicocele

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Study question: Can varicocele repair contribute to improve semen parameters and pregnancy rate compared with medication or observation in infertile males with clinical varicocele?
Summary answer: There is a possibility that varicocelectomy (microsurgical sub-inguinal varicocelectomy at unilateral side or laparoscopic varicocelectomy at both sides) can improve pregnancy rate rather than semen parameters.
What is known already: Clinically palpable varicoceles have been clearly associated with infertility. Some studies show an improvement in semen parameters and fertility after repair of varicoceles.
Study design, size, duration: This was a retrospective study that included 321 infertile male patients with varicocele (Grade 1–3) with semen parameters of normozoospermia, oligozoospermina and/or asthenozoospermia in 2 hospitals from September 2007 to August 2015.
Participants/materials, setting, methods: Surgery group was 134 patients who underwent varicocelectomy, non-Surgery group was 187 patients who took medication or observation. Patients were assessed by clinical evaluation, semen analysis (before treatment and at 2, 4, 6, 9, 12 months following) and pregnancy results by medical records and questionnaires. Patients who had not come to hospital more than 6 months without any information and did not answer the questionnaire have been excluded from pregnancy rate analysis.
Main results and the role of chance: The semen parameters have not improved with the Surgery group compared with the non-Surgery group. Pregnancy rate is 32.5% (27/83) for patients in the Surgery group and 18.8% (19/101) in the non-Surgery group. Pregnancy rate increased with operation treatment ($p < 0.05$). In the Surgery group, 7 patients conceived naturally, 6 patients by intrauterine insemination (IUI) and 13 patients by *in vitro* fertilization/intracytoplasmic sperm injection (IVF/ICSI) while 4 patients had miscarriages. As for the non-Surgery group, 6 patients conceived naturally, 2 patients by IUI, 11 patients by IVF/ICSI while 5 patients had miscarriages.
Limitations, reasons for caution: This was a small retrospective study. Female factors were not considered but there was no significant difference in their age between 2 groups.
Wider implications of the findings: Varicoceletomy can improve the quality of sperms which are not reflected in semen analysis. Therefore it can increase pregnancy rate and contribute to a decrease in the number of patients who need assisted reproductive technology.
Trial registration number: None.

P-066 The effect of prolonged sexual abstinence period in male on fertilization and pregnancy outcomes in fresh embryo transfer after intracytoplasmic sperm injection

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Study question: Does prolonged sexual abstinence period in male affect fertilization and pregnancy outcomes in fresh embryo transfer after intracytoplasmic sperm injection (ICSI)?
Summary answer: Our study showed that prolonged sexual abstinence period in male does not influence on fertilization and pregnancy outcomes in fresh embryo transfer after ICSI.
What is known already: A short or long sexual abstinence period is associated with incorrect semen analysis results, along with intrauterine insemination using sperm of short abstinence period is correlated with higher pregnancy rates. Previously reported more than eight days abstinence period has increased sperm DNA fragmentation; however, the World Health Organization (WHO) recommended that abstinence period that surpasses 8 days is not related to increased the proportion of aneuploidy embryos.
Study design, size, duration: A retrospective cohort study in 322 ICSI cycles was conducted between January 2013 and November 2015 at Agaon Fertility Clinic. We compared rates of fertilization, top-quality embryos, and pregnancy outcomes between the two groups.
Participants/materials, setting, methods: Participants were divided into two groups: G-A (2–7 days, $n = 225$) and G-B (≥8 days, $n = 97$). The sexual abstinence period was identified before collecting semen samples. The semen analysis was performed according to the manual guideline of the WHO (2010). The cycles with poor responder, advanced maternal age (≥38 years), frozen sperm, oligoasthenoteratozoospermia, and surgically retrieved sperm were excluded.
Main results and the role of chance: There were no significant differences between G-A and G-B regarding the patients' characteristics, the female age (33.8 ± 1.8 vs. 33.7 ± 2.5, $p = 0.0710$), the male age (36.0 ± 4.0 vs. 36.6 ± 3.8, $p = 0.186$), the number of retrieved oocytes (11.6 ± 5.1 vs. 11.1 ± 4.5, $p = 0.377$), the number of transferred embryos (2.3 ± 0.7 vs. 2.3 ± 0.6, $p = 0.998$), fertilization rate (75.9% vs. 79.2%, $p = 0.077$) and the rate of top-quality embryos on day 3 (12.5% vs. 12.5%, $p = 0.965$), respectively. The biochemical pregnancy (43.1% vs. 45.4%, $p = 0.709$), clinical pregnancy (36.9% vs. 35.1%, $p = 0.753$), and ongoing pregnancy (33.8% vs. 29.9%, $p = 0.496$) rates per cycle did not significantly differ in both G-A and G-B.
Limitations, reasons for caution: The number of analyzed G-B cycles was relatively small compared with the G-A. We were not limited after the sexual abstinence period of more than 8 days and male age. Further studies are required large data samples of this size.
Wider implications of the findings: Although both a strong correlation between sexual abstinence period and sperm parameter, we haven't established a connection among fertilization, pregnancy outcomes and prolonged sexual abstinence period in fresh embryo transfer after ICSI. The reason might be because a viable sperm with normal morphology and progressive motility were selected during ICSI.
Trial registration number: Not applicable.

P-067 Dynamic transcriptional profiles of human testis-specific actin capping protein β3 and its possible implication of male infertility

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Study question: Does actin capping protein (CP) β3 play an important role in male infertility?
Summary answer: Human CPβ3 is supposed to be essential, as is expressed in testis dynamically changing its localization during spermatogenesis and possibly has implication of male infertility.
What is known already: Testis-specific α subunit of CP named CPα3 was identified in human testis previously. CPα3 was localized in the tail and postacrosomal region of sperm head. N-ethyl-N-nitrosourea (ENU)-induced mutation in CPα3 gene in mouse was shown to induce malformation of sperm head and to be responsible for male infertility. On other hand, CPβ3 which is supposed to be a heterodimeric counterpart of CPα3 has been described in mouse or bovine in several literatures. However, human CPβ3 has been neither characterized nor reported about the association with male infertility.
Study design, size, duration: To confirm the existence of CPβ3 in human testis, fresh semen samples from fertile male were observed. To investigate the protein expression during spermatogenesis, ten human cryopreserved samples were observed retrospectively, which were obtained by testicular sperm extraction from men diagnosed as obstructive azoospermia. To compare the protein expression of fertile and infertile male, cryopreserved sperm were investigated retrospectively. This study was approved by the ethical committee and informed consent was obtained from each volunteer.
Participants/materials, setting, methods: The tissue specific expression of CPβ3 was investigated by RT-PCR and Western blot analysis. The relationship between CPα3 and CPβ3 was analyzed by coimmunoprecipitation (Co-IP) assay with a use of recombinant protein tagged with EGFP or mRFP. The transcriptional profiles of CPα3 and CPβ3 during spermatogenesis were examined by immunohistochemical analysis using human spermatogenic cells. The expression patterns of CPα3 and CPβ3 in sperm from infertile male were observed and compared by immunohistochemical analysis.
Main results and the role of chance: RT-PCR showed that mRNA of human CPβ3 was expressed exclusively in testis. Western blot analysis successfully detected human CPβ3 with anti-bovine CPβ3 antibody. Co-IP assay with recombinant protein showed that CPα3 and CPβ3 form a protein complex.

In each step of cells during spermatogenesis, the localization of CPβ3 dynamically changed. In spermatogonia, CPβ3 showed slight signal in cytoplasm. Mainly from spermatocytes, the expression of CPβ3 was conspicuous and the localization of CPβ3 dynamically migrated from cytoplasm to acrosome or postacrosomal region of sperm head. On the other hand, the localization of CPα3 was completely identical to that of CPβ3 in every step of spermatogenic cells. In mature spermatozoa, both CPα3 and CPβ3 were accumulated at postacrosomal region and slightly at midpiece of tail. While the localization of CPα3 and CPβ3 was homogeneous in most of the spermatozoa in fertile male, heterogeneous or lack of staining of CPα3 and CPβ3 were identified in infertile male. The complex of CPα3 and CPβ3 seemed to play an important role in spermatogenesis and possibly have association with male infertility.
Limitations, reasons for caution: Because of the difficulty of collecting fresh samples of human testis, we used cryopreserved samples of TESE. To examine the interaction of spermatogenic cells or localization in seminiferous tubules, fresh testis sample of healthy male is better to be used ideally.
Wider implications of the findings: The altered expression of CPα3 and CPβ3 can be not only a cause of male infertility but also a prognostic factor for the result of assisted reproductive technologies (ART). They can be a useful biomarker to diagnose infertile males who are not azoospermia but have difficulties in ART.
Trial registration number: NA.

P-068 Venous blood gases of varicocele veins: correlation with testicular blood flow and semen quality in varicocele patients

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Study question: Changes in blood gas patterns of varicocele veins may affect testicular blood flow and semen quality.
Summary answer: Internal spermatic veins of patients had higher pO2, sO2, lower pH and HCO3 as compared to peripheral veins. External spermatic veins were less affected.
What is known already: Varicocele is known to be associated with infertility and sperm disorders. There are limited numbers of studies where venous blood gases of the varicocele veins of testes were determined.
Study design, size, duration: Consecutive varicocele patients (aged 20–45 years), undergoing left microsurgical varicocelectomy, were included in the study during January 2013 to August 2015.
Participants/materials, setting, methods: Twenty-seven varicocele patients, were included in the study. The pH, partial pressure of oxygen (pO_2), oxygen saturation (sO_2), partial pressure of carbon dioxide (pCO_2) and bicarbonate (HCO_3) levels of varicocele veins were determined in Internal spermatic, external spermatic and peripheral veins, while semen quality parameters were determined before surgical intervention. Peak systolic velocity (PSV) and resistive index (RI) of sub capsular and Intra parenchymal branches of the testicular artery were measured using scrotal CDUS.
Main results and the role of chance: Results revealed that the pH was lower ($p < 0.001$) in the internal spermatic vein ($p < 0.01$) compared with the external spermatic and peripheral veins. The pO_2 and sO_2were higher ($p < 0.001$) in the internal spermatic vein ($p < 0.01$) compared with peripheral vein. However, HCO_3 content was lower ($p < 0.05$) in both varicocele veins compared with the peripheral vein. Whereas, the pCO2 was similar ($p > 0.05$) in all three veins. The pO_2 of internal spermatic vein had negative correlation ($r = -0.42$; $p < 0.05$) with PSV of the intra parenchymal arteries. No such correlation was observed for external spermatic vein or peripheral vein. There were non-significant weak correlations between various parameters of VBG of both varicocele veins with semen quality parameters in varicocle individuals. In conclusion, internal spermatic veins had higher oxygen level and lower pH and bicarbonate levels whereas external spermatic veins had similar pattern of blood gases to the peripheral veins in the varicocele patients.
Limitations, reasons for caution: Further studies are required to confirm our findings.
Wider implications of the findings: The apparent difference of VBG analysis between varicocele veins and peripheral veins may be due to the presence of arterio venous communications or some other mechanism that needs further investigation.
Trial registration number: This is not a clinical trial.

P-069 Differential chromatin incorporation of CREM, ACT and BRDT testis-specific transcription factors in normal and impaired human spermatogenesis

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Study question: What is the potential epigenetic role of transcription regulators (CREM, ACT and BRDT) in spermatogenesis impairment and male infertility?
Summary answer: Decreased expression of CREM, ACT and BRDT transcription factors parallel to decreased incorporation of them into promoters of spermatogenesis-specific genes were correlated with spermatogenesis impairment.
What is known already: During spermatogenesis chromatin of sperm undergoes extensive epigenetic remodeling to be compacted and packaged. Several studies have shown that impairment in this process is associated with infertility in men. Some genes such as cAMP response element modulator (CREM) and Bromodomain testis-specific protein (BRDT) are master regulators for expression of famous chromatin condensing factors of spermatogenesis, transition proteins (TNPs) and protamines (PRMs).
Study design, size, duration: Through ART procedure, testes tissue samples were collected from azoospermic infertile men referred to Royan Institute. Consent was obtained from each patient according local ethical approval. Based on pathological features, tissue samples divided into following three groups: complete maturation arrest, Sertoli cell only syndrome, and hypospermatogenesis as positive control (at least 30 samples in each group).
Participants/materials, setting, methods: Relative expression of CREM, ACT (activator of CREM in testis) and BRDT were evaluated by qRT-PCR. Also, ChIP-real time PCR was performed to evaluate the incorporation of CREM, ACT and BRDT transcription factors into regulatory regions of TNPs and PRMs genes.
Main results and the role of chance: Relative expression profile of CREM, ACT and BRDT showed significant decrease in complete maturation arrest and Sertoli cell only syndrome groups compared to hypospermatogenesis group ($p < 0.05$) as well as TNPs and PRMs. The results of expression confirmed by ChIP data revealed decreased incorporation of CREM, ACT and BRDT as transcription regulators of spermatogenesis into regulatory regions of TNPs and PRMs in both groups with spermatogenesis impairment vs. positive control ($p < 0.05$).
Limitations, reasons for caution: The study population could be expanded and the interaction between other transcription factors/regulators is required to be investigated.
Wider implications of the findings: The findings of this study imply significant association between altered levels of transcription regulators of spermatogenesis with impairment of spermatogenesis and male infertility.
Trial registration number: NA.

P-070 IVF outcomes and morphokinetics of embryos originating from testicular spermatozoa of men with follicle stimulating hormone receptor (FSHR) gene polymorphism

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Study question: Does the polymorphism in FSHR gene affect the IVF success rates and early development of embryos obtained after oocyte fertilization by testicular sperm?
Summary answer: Significant differences in embryo morphokinetics, IVF outcomes were observed in dependence on peculiarities of sperm finding but not on the presence of FSHR alternative alleles.
What is known already: The data concerning the effect of FSHR gene polymorphism on male infertility are controversial. Some studies have shown associations of genetic disturbances in FSHR with azoospermia, sperm aneuploidy, spermatogenesis impairment and serum FSH level, while other researches have not demonstrated any correlation between alternative variants of FSHR and sperm abnormalities or FSH concentration. Moreover, no results regarding possible influence of FSHR gene polymorphism on embryo morphokinetics in case of oocyte fertilization by testicular spermatozoa of men with azoospermia have been reported to date.
Study design, size, duration: Retrospective study (2012–2015) of morphokinetics of 576 embryos obtained after testicular (n = 54) or donor ejaculated (n = 24) sperm injections in oocytes of fertile women or women with tubal factor infertility (31.6 ± 3.9 years old) was conducted. Fertilization, blastulation and pregnancy rates, percent of embryo abnormal cleavage rate were assessed. Testicular spermatozoa were extracted during TESA/TESE in case of obstructive azoospermia (OA) or microTESE in case of non-obstructive azoospermia (NOA).
Participants/materials, setting, methods: The three groups of patients were compared: 1) men with azoospermia and normal FSHR genotype (n = 33); 2) men with azoospermia and FSHR gene polymorphism (n = 21); 3) sperm donors (control, n = 24). Embryo morphokinetic parameters (2pn fading, 2–8 cell divisions, intervals between divisions, direct and reverse cleavage, blastocyst formation) were analyzed using time-lapse monitoring system (PrimoVision). Thr307Ala and Asp680Ser FSHR polymorphisms were determined by Taqman assays on the ABI PRISM 7500 real-time PCR system.
Main results and the role of chance: Among 21 patients with azoospermia and FSHR gene polymorphism 17 men were the carriers of polymorphism in the both alleles of FSHR. The fertilization rate (79.6 vs 81.0%), percent of the top quality embryos (60.6 vs 42.9%), blastocysts formation (50.1 vs 47.2%) and clinical pregnancy rate (36.0 vs 30.8%) did not statistically differ between the groups 1 and 2. However, these parameters were significantly lower, than in control (91.1, 81.0, 67.6 and 48.8%, respectively, $p < 0.05$). The morphokinetic parameters also were similar in the both groups with azoospermia. With that period of pronucleus faiding, synchrony in division from 3 to 4 cells (s2) and time of division to 8 cells were longer, but time of division to 3, 5 cells and duration of the 2nd cell cycle (cc2) were shorter comparing with the control ($p < 0.05$). Direct cleavage to more than 2 cells was significantly higher in groups 1 and 2 (33.0 and 29.3%) vs control (16.3%, $p < 0.05$).
Limitations, reasons for caution: The study included only those patients who have been found at least a few motile sperm during TESA/TESE or microTESE (the spermatozoa were found in 54 men from 70). We did not analyze embryos separately for OA and NOA.
Wider implications of the findings: In the present study no spermatozoa were found in 12 carriers of polymorphism in FSHR gene (75% from all cases of sperm absence). The futher investigations of association between the treatment failures with alternative alleles in FSHR gene should be continued to develop the necessary therapy for stimulation of spermatogenesis.
Trial registration number: No registration number.

P-071 Exploring the role of the nuclear receptor coactivators in murine Sertoli cell

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Study question: What is the role of nuclear receptors (NR) coactivators Ncoa1 and Ncoa2 in Sertoli cells?
Summary answer: Ncoa1 and Ncoa2 are instrumental to the maintenance of spermatogenesis by acting in Sertoli cells.
What is known already: Genetic analyses in mouse have shown that NR coactivators Ncoa1 and Ncoa2 have individually redundant functions in male reproduction function. These results were obtained by classical gene knock-out study. However, compound Ncoa1-/- Ncoa2-/- double null mutants die at birth precluding any study of the effects of Ncoa1/Ncoa2 simultaneous ablation in the adult male testes.
Study design, size, duration: To study the role of Ncoa1 and Ncoa2in Sertoli cells, mice expressing the Cre transgene expressed under the control of the anti-Müllerian hormone (Amh) promoter were crossed with mice bearing loxP-flanked alleles for both Ncoa1 and Ncoa2. The Amh-Cre transgene yields an efficient excision of loxP-flanked genes in all Sertoli cells as early as embryonic day 15.5.
Participants/materials, setting, methods: To study the role of Ncoa1 and Ncoa2in Sertoli cells, mice expressing the Cre transgene expressed under the control of the anti-Müllerian hormone (Amh) promoter were crossed with mice bearing loxP-flanked alleles for both Ncoa1 and Ncoa2. The Amh-Cre transgene yields an efficient excision of loxP-flanked genes in all Sertoli cells as early as embryonic day 15.5.
Main results and the role of chance: Invalidation both Ncoa1 and Ncoa2 in murine Sertoli cells is responsible for a massive desquamation of immature germ cells associated with an increase of lipids stored in Sertoli cells and the formation of giant multinucleated spermatids. These abnormalities are identical, in their morphological appearance, to those observed upon inactivating either Ncoa1 or Ncoa2 in the whole organism, thereby demonstrating that all the functions that Ncoa1 exert in reproduction are carried out in Sertoli cells.
Limitations, reasons for caution: These results were obtained in mice and therefore the relevance on human spermatogenesis remains to be demonstrated.
Wider implications of the findings: Increase in Sertoli cell lipid stores, loss of immature germ cells, and formation of giant multinucleated spermatids are commonly detected in testes of elderly men, suggesting that deficiencies in molecular pathways involving NCOA1 and NCOA2 in human Sertoli cells could participate in testicular senescence.
Trial registration number: NA.

P-072 Severe testicular microlithiasis in Ultrasonographic (US) images indicates the micro-obstruction in seminiferous tubule and may predict high sperm retrieval rate in non-obstructive azoospermia (NOA) patient

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Study question: Can US finding of severe testicular microlithiasis predictive successful sperm retrieval?
Summary answer: Severe testicular microlithiasis in US images may predict high sperm retrieval rate in NOA patient.
What is known already: Testicular microlithiasis has been reported to be infrequent condition associated with testicular tumors or testicular deficiencies. However the mechanisms of testicular microlithiasis or sperm retrieval rate of micro-TESE in patients with NOA with testicular microlithiasis are not well known.
Study design, size, duration: Echo patterns of the testis were observed by 10~14MHz linear probe, of the 596 patients who visited our clinic for male infertility from July 2003 to August 2015. Endocrinological measurements and US images of the testis were obtained before surgery, and compared with the results of micro-TESE.
Participants/materials, setting, methods: We evaluated 596 patients who underwent microTESE. US findings were compared with testis volumes, microscopic findings (HE stain), FSH, and sperm recovery rate. US analysis was performed using a 10- or 14-MHz linear array transducer. To optimize US images for visualization of the seminiferous tubules, gain and contrast were appropriately adjusted with graphic software.
Main results and the role of chance: Severe testicular microlithiasis (high echoic lesion with acoustic shadowing at surfaces of testis) were observed in 6 (1.0%) out of 596 NOA patients. Median age was 39 years old (range: 32–62 years old), and on physical examination, testis volume was 2–11 ml (average 4.21ml), serum follicle stimulating hormone value was high (13.0–36.3mIU/ml; average 26.3mIU/ml). All of 6 patients with severe testicular microlithiasis underwent microTESE for treatment of azoospermia, and sperm retrieval was successful in all patients with severe testicular microlithiasis. Many calcifications with layered structure were observed in every seminiferous

tubule tissue in microscopic findings, and Johnsen's score count was 1.55–6.50 (average 4.21).

Limitations, reasons for caution: Severe testicular microlithiasis was observed in only 6 patients out of 596 NOA patients in our clinic. However, to discuss about sperm retrieval rate in patients with severe microlithiasis, more number of patients should be included for the future study.

Wider implications of the findings: Severe testicular microlithiasis indicates the micro-obstruction in seminiferous tubule in NOA patient. When severe testicular microlithiasis was observed in NOA patients in US analysis, its finding may be the predictive factor of successful sperm retrieval in NOA patient.

Trial registration number: Not required.

P-073 Association of varicocele and classical sperm characteristics with antioxidant enzymatic activity – superoxide dismutase and glutathione peroxidase, selenium, sperm DNA fragmentation and membrane mitochondrial potential

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Study question: Oxidative stress (OS) has been mentioned as being a cause of male infertility. Are the OS markers, actually used, useful in diagnosing sperm pathology ?

Summary answer: Beside lower SOD and GPx in men with varicocele, no association was retrieved between DNA fragmentation and MMP and other markers of seminal oxidative stress.

What is known already: Seventy years have passed after the discovery of detrimental effect of reactive oxygen species (ROS) on sperm and its possible repair after addition of antioxidant enzymes.

Low ROS levels are normal whereas seminal oxidative stress (OS) with ROS produced in excess, can impair sperm.

High antioxidant enzymatic scavengers (SOD, GPx), total antioxidant activity and selenium are characteristic of normal sperm.

Technology fails to propose reliable tests to detect accurately oxidants and antioxidants and their negative effects on sperm.

Beside sperm standard characteristics, DNA fragmentation and MMP distinguish normal sperm from abnormal and predict natural conception.

Study design, size, duration: Eighty eight males from infertile couples were consecutively enrolled at the outpatient clinic at the University Medical Center Ljubljana over a period of two years. Aoospermics were excluded.

Participants/materials, setting, methods: Men underwent clinical examination: medical history of testicular mal descent, testicular volume and palpable varicocele. Sperm analysis was performed according to WHO 1999 guidelines. The percentage of sperm with DNA fragmentation was determined by TUNEL method, MMP was determined by means of $DiOC_6(3)$. TAC, **s**uperoxide dismutase (SOD), glutathione peroxidase (GPx) and selenium were determined by spectrophotometric assays.

Main results and the role of chance: The classical sperm characteristics (concentration, motility, morphology) were related to DNA fragmentation negatively and to MMP positively (all $p < 0.001$). GPx and SOD were negatively related to the presence of a clinical varicocele ($p < 0.001$ and $p = 0.031$, respectively). Selenium was associated with sperm concentration and morphology ($r = 0.236$, $p = 0.038$ and $r = 0.257$, $r = 0.024$, respectively). Selenium was also related to GPx ($r = 0.231$, $p = 0.043$) and SOD ($r = 0.534$, $p < 0.001$).

This study does not retrieve clear relationships between OS markers, standard sperm characteristics, DNA fragmentation and MMP.

Limitations, reasons for caution: Limited number of participants; may be not the adequate OS tests selected.

Wider implications of the findings: Necessity to develop basic research on oxidative stress and to develop screening tests.

Trial registration number:/

P-074 The common DEFB126 NM_030931.3:c.314_315delCC genetic variant: an indicator for treatment and donor selection in subfertile couples?

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Study question: Does the common β-defensin 126 (*DEFB 126*) genetic variant c.314_315del affect the success rate of donor intra-uterine insemination (D-IUI)?

Summary answer: The results show no difference in the pregnancy rate between the three different variants of *DEFB126* (ins/ins; del/del; del/ins).

What is known already: The *DEFB126* variant NM_030931.3:c.314_315delCC has been suggested to modify immunoprotection and efficient movement of sperm in the female reproductive tract. In a study of 509 Chinese couples, odds of pregnancy were significantly reduced (to 60%) when the male had the del/del genotype. *In vitro,* there was 84% reduction in the rate of penetration of a hyaluronic acid (HA) gel by sperm from del/del males. The authors suggested two mechanisms: impaired penetration of cervical mucus and reduced sperm-oocyte adhesion.

Study design, size, duration: Retrospective cohort study of 126 women with 205 D-IUI cycles from 37 semen donors, who underwent D-IUI between 2013 and 2015. D-IUI was selected both to remove the barrier of cervical mucus, and to determine whether this test could improve donor selection.

Participants/materials, setting, methods: Participants included all women who commenced D-IUI treatment in our fertility center. Exclusion criteria included the presence of female factors and women older than 36. Pregnancy was defined as β-HCG > 15 IU/ml; pregnancy rate was estimated for each genotype group of *DEFB126* (ins/ins; del/del; del/ins). The *DEFB126* variant was tested by PCR and capillary electrophoresis on the donors' DNA stocked in the genetic laboratory.

Main results and the role of chance: In all there were 58 pregnancies in the 205 D-IUI cycles (28.3%). There were 14 pregnancies in the group ins/ins (30.4%); 21 in the group del/del (30.8%) and 23 in the group del/ins (25%). The pregnancy rate of the three groups was not statistically different.

Limitations, reasons for caution: This pilot study was performed retrospectively, and only on sperm donors. All participants were Caucasian, in contrast to the original study.

Wider implications of the findings: As we studied IUI cycles, our results suggest that any effect of the del/del genotype is related to cervical mucus. Therefore IUI would be the treatment of choice in couples with del/del males. Furthermore, *DEFB126* analysis is useful in the workup of infertile couples but not in sperm donors selection.

Trial registration number: None.

P-075 Reconsidering of human round spermatid injection into the oocyte (ROSI)

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Study question: Can an improved ROSI be considered a worthy clinical treatment despite the fact that conventional ROSI has been recognized as being ineffective for non-obstructive azoospermia?

Summary answer: In this study ROSI fully proved to be effective clinically when there is proper identification of round spermatid and oocyte activation.

What is known already: To date, the application of ROSI in clinical IVF has had disappointing results due to difficulties in accurate identification of round spermatids among other round spermatogenic cells and insufficient oocyte activation. Incomplete male-specific DNA methylation imprinting in round spermatids might be the cause of the insufficiency of ROSI. It is believed that existence of round spermatid is associated with testicular spermatozoon. That is no testicular spermatozoa means no round spermatid.

Patients who may be candidates for ROSI should receive careful and thorough pretreatment counseling to ensure they are clearly informed of the limitations and potential risks of the procedure.
Study design, size, duration: A total of 90 non-obstructive azoospermic men whose first Micro-TESE conducted by andrologists showed no testicular spermatozoa or late staged spermatids but had round spermatids found at our hospital and received ROSI from September 2011 to December 2014 participated in this study. After approval of the Institutional Review Boards of the Saint Mother Obstetrics and Gynecology Clinic. This study was registered and adhered to International Committee of Medical Journal Editors criteria.
Participants/materials, setting, methods: Round spermatids, cytologically selected after thawing, were injected into ooplasm which was activated 10 minutes before the injection by electrical stimulation with an alternating current pulse of 2V/cm for 8s + direct current pulse of a single 1.2kV/cm for 99μs. We examined pattern of Ca^{2+} oscillation in oocytes after electrical stimulation and ROSI plus electrical stimulation to know the efficacy of the oocyte activation.
Main results and the role of chance: The percentages of occurrence of 1PN, 2PN and 3PN after electrical stimulation were 80% (8/10), 20% (2/10) and 0 respectively. Fertilization and rate to develop over 4 cell stage were 58.8% (590/1004), 50.6% (508/1004) respectively. The pregnancy rate per transferred cycle, miscarriage rate and birth rate in fresh embryo transfer cycles and freezing-thawed transfer cycles were [16.7%(26/156), 65.3%(17/26), 5.8%(9/156)], [23.9%(16/67), 50.0%(8/16), 11.9%(8/67)] respectively. No abnormal karyotype and genomic imprinting abnormalities (IGF2, H19, SNRPN) were identified in any of the newborn babies. All of 6 female and 13 male babies are healthy and no serious physical or cognitive disorders have been reported so far. Although round spermatid injection alone could induce small Ca^{2+} oscillations, ROSI with prior electrical stimulation showed effective in inducing repetitive, large Ca^{2+} oscillations.
Limitations, reasons for caution: The limitation of this clinical study concerns the small size of the study group and electrical stimulation for oocyte activation could not be the optimal one judging from the high percentage of miscarriages and immatured centrosome. The inefficient demethylation and remethylation of DNA after ROSI might be a prime concern.
Wider implications of the findings: This study presents evidence that ROSI has a high potential to help many non-obstructive azoospermic men whose most advanced spermatogenic cells are round spermatids. After confirmation of the safety of ROSI, it could be chosen as a first line treatment instead of ICSI using dead or anomalous spermatozoa.
Trial registration number: UMIN Clinical Trials Registry UMIN000006117.

P-076 Y chromosome microdeletion and spermatogenesis in Japanese men due to spermatogenic dysfunction

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Study question: What is the frequencies of microdeletions of AZFa, AZFb, AZFc and the sperm retrieval rate by TESE in azoospermia due to spermatogenic dysfunction from Japanese population?
Summary answer: The frequency of Y chromosome microdeletions in infertile men from Japan was comparable to the frequency reported in other countries and regions.
What is known already: Genetic factors cause about 15% of male infertility. Azoospermia factors: AZFa, AZFb (P5/proximal P1), and AZFc (b2/b4) present on Yq are most important for spermatogenesis.
Study design, size, duration: This study is Retrospective analysis for azospermic patients who conducted TESE from April 2007 to December 2014.
Participants/materials, setting, methods: We included 980 azoospermia due to spermatogenic dysfunction (ASD) men in present study, belonging to the age group of 20–51 years. For each patient genomic DNA was extracted from peripheral blood using, the QIAamp DNA mini extraction kit. Y Chromosome Deletion Detection System, Version 2.0 was used for Y chromosome deletion detection system. A single surgeon performed microdissection testicular sperm extraction (TESE) for all enrolled ASD patients. The pathological diagnosis of resected testicular specimen was examined.
Main results and the role of chance: The overall prevalence of Y chromosome microdeletions in infertile men was 8.1% (79/980). Prevalence of Y chromosome microdeletions in AZFa, AZFb, AZFabc, AZFbc (P5/distal P1 or P4/distal P1) and AZFc are 0.1%, 0.8%, 0.7%, 2.0% and 4.4%, respectively. Microdissection TESE failed in all patients with AZFa, AZFb, AZFabc, and AZFbc. Sperm were retrieved in 28/43 AZFc deleted patients (62.2%). The presence of an AZFc deletion was associated with significantly increased of sperm retrieval when compared with the 33.0% retrieval rate in idiopathic nondeleted azoospermic men who consecutively underwent microdissection TESE at our institution during the study period.
Limitations, reasons for caution: Study data were obtained retrospectively, which might have affected the quality of the data. Furthermore, the sample size was too small to draw definite conclusions from the results. We need to accumulate additional data from similar cases.
Wider implications of the findings: None.
Trial registration number: None.

P-077 Relationship between serum oxidative stress/antioxidant power in infertile men and age, parameter of sperms or ART result

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Study question: How serum oxidative stress (OS) and antioxidant power (AOP) in infertile men influence parameters of sperms and outcome of ART?
Summary answer: There were no correlations between OS/AOP and parameters of sperms. The normal fertilization rates in the elevated OS and lower AOP groups were significantly lower.
What is known already: OS is a consequence of an imbalance between the production of reactive oxygen species and the body's antioxidant defense mechanisms. OS has also been implicated in the pathogenesis of many human diseases such as cancer, diabetes and motor neuron disease. OS can lead to gamete damage, deformity and eventually infertility.
Study design, size, duration: The value of serum OS and AOP in 225 infertile men were measured between March and October of 2015. Among the 225 men, sperm parameters of the 48 men were measured on the day of oocyte retrieval and the ART results of the 83 males were assessed.
Participants/materials, setting, methods: D-ROMs (reactive oxygen metabolites) and BAP (biological anti-oxidant potential) in the serum were measured. Correlation between serum d-ROMs or BAP and sperm DNA fragmentation index, sperm concentration or sperm motility rate were observed. The ART results were compared in terms of the value of men's serum d-ROMs and BAP.
Main results and the role of chance: There was no correlation between the men's age and the levels of d-ROMs while the level of BAP decreased with advancing age. There were no correlations between serum d-ROMs or BAP and sperm DNA fragmentation index, sperm concentration or sperm motility rate. The normal fertilization rate in the men's d-ROMs elevated group was significantly lower than that in the d-ROMs normal group. There were no significant differences in the good-quality embryo rate on day 3 and the blastocyst formation rate between the two groups. The normal fertilization rate in the men's BAP lower group was significantly lower than that in the normal group. There were no significant differences in the good-quality embryo rate on day 3 and the blastocyst formation rate between the two groups.
Limitations, reasons for caution: Sample of this study was small. At this study, the levels of OS and AOP in the seminal plasma weren't measured.
Wider implications of the findings: The value of serum OS and AOP did not influence sperm DNA fragmentation index, sperm concentration or sperm motility rate. The normal fertilization rate in the men's serum OS elevated group was significantly lower and the normal fertilization rate in the men's serum AOP lower group was significantly lower.
Trial registration number: N/A.

P-078 Influence of monotherapy with darunavir-ritonavir on semen quality and on the presence of HIV in the semen of HIV+ patients

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Study question: Is monotherapy with darunavir-ritonavir as safe as triple therapy in relation to the presence of HIV in semen, and is semen quality maintained?
Summary answer: Changing triple therapy to monotherapy does not alter semen quality and is as safe as triple-therapy in relation to the presence of HIV in semen.
What is known already: Opinions differ regarding the effect of HIV infection and of the various treatments used on the semen parameters of these patients. Moreover, studies have confirmed that patients with undetectable viral load and no sexually transmitted disease do not transmit the virus sexually. However, little research has been conducted into the viral load in semen among patients receiving monotherapy with protease inhibitors, and the effect of new therapies, facilitating medication adherence, on the semen quality of HIV patients remains unknown.
Study design, size, duration: Observational study in HIV patients with undetectable viral load (<20 copies) in blood plasma under triple therapy, including 2 nucleos(t)ide analogues, and who are candidates for monotherapy with a boosted protease inhibitor (darunavir-ritonavir). The variables analysed were age, CD4/mL, nadir CD4, type of triple therapy, semen viscosity, appearance, liquefaction, volume, pH, sperm concentration, progressive and total motility, vitality, andrological history and reproductive history.
Participants/materials, setting, methods: For each patient, two separate semen samples were analysed, at 15-day intervals, before discontinuing triple therapy, and another two samples were analysed after 48 weeks of monotherapy with darunavir-ritonavir. All semen samples were processed and evaluated in accordance with WHO (2010) recommendations. The technique used to quantify HIV-1 RNA in the final sample processed was real-time PCR, an adaptation of the HIV-1 COBAS Ampliprep/Taqman assay quality of HIV patients remains unknown.
Main results and the role of chance: A total of 28 patients, with a mean age of 41 ± 9.13 years (22–55), were included. The percentages of patients with values above the WHO 2010 lower reference limit for all semen parameter values were similar before and after the change to monotherapy: normozoospermia (57.1% vs. 61.9%), oligoteratozoospermia (7.1% vs. 9 6%), oligoasthenozoospermia (7.1% vs. 4.8%), oligoteratoasthenozoospermia (10.7% vs. 4.8%), teratozoospermia (14.3% vs. 4.8%), asthenozoospermia (0% vs. 9 6%), oligozoospermia (3.6% vs. 4.8%). The positive viral load in seminal fluid in patients with triple therapy, in the first semen sample, was 14.81%, ranging from 35 to 1210 copies/mL. The positive viral load in seminal fluid in patients with triple therapy in the second sample was 4.55% with 399 copies/mL. The corresponding values in monotherapy with darunavir-ritonavir were 3.13% (positive viral load: 139 copies/mL) in the first sample and 0% in patients with positive viral load in the second sample. No relationship was observed between the presence of HIV in semen and the type of therapy.
Limitations, reasons for caution: These findings need to be verified in studies with larger numbers of patients. ence of HIV in semen.
Wider implications of the findings: The results obtained could facilitate the response to the reproductive wishes of seronegative partners of HIV-positive patients.
Trial registration number: No trial registration number.

P-079 Recipient nationality and marital status in relation to choice of ART and donor type

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Study question: Does recipient nationality and marital status affect the preference for anonymous/non-anonymous donors and does recipient nationality relate to choice of ART?
Summary answer: The preference for non-anonymous vs. anonymous donors is influenced by marital status and nationality of the recipient. The latter also influences the choice of ART.
What is known already: Many countries regulate use of anonymous/non-anonymous gamete donors, but often in conflict with the recipient's preferences. Views concerning the recipient's right to autonomy and interest of the child vary greatly both between individuals and national laws, e.g., certain countries only allow treatment for heterosexual couples and with anonymous donor sperm. In contrast, other countries allow for both anonymous and non-anonymous donors and treatment regardless of marital status. These differences induce some recipients to seek alternative solutions either abroad, i.e., CBRC, home insemination or unauthorized suppliers.
Study design, size, duration: A multiple-choice questionnaire was e-mailed to recipients with the purpose of investigating preferences and experiences of these recipients. The current analysis emphasizes the recipient's opinion with respect to donor anonymity status and recipients choice of ART as a function of marital status and nationality. 1463 replies were received.
Participants/materials, setting, methods: Recipients of donor sperm from the sperm bank Cryos International - Denmark received a multiple-choice questionnaire by e-mail and responded from September 2014 until October 2015. Results were tested for statistical significance using the Chi-square test, and, hence, comparisons were accomplished employing nationalities harboring most of the responders, i.e., Germany, United Kingdom, France, Italy, Netherlands, Sweden, and Denmark.
Main results and the role of chance: The frequency of people living in a homosexual relationship using donor sperm was significantly higher in Germany, United Kingdom, France, and Italy compared to Denmark, Netherlands, and Sweden, where the marital status of the largest group of recipients were single. Furthermore, recipients who were single or in a homosexual relationship, did find it more important that there was a wide selection of non-anonymous donors than those living in a heterosexual relationship, who found it more important that there was a wide selection of anonymous donors, than the other groups. Furthermore, the survey revealed that the majority of the recipients from all seven nationalities found it important that there was a wide selection of non-anonymous donors where recipients from the Netherlands found it even more important than recipients from the remaining countries.

Recipients from the various countries were also asked for their choice of treatment, including home insemination, IUI, IVF and ICSI. Interestingly, the survey revealed that recipients from Germany, United Kingdom, France, and Italy presented the highest frequency with respect to home inseminations whereas recipients from Denmark revealed the lowest. The Netherlands and Sweden were located between those two groups with respect to home insemination frequency.
Limitations, reasons for caution: Only a minority responded to the questionnaire. It is unknown if all groups are equally likely to respond. Recipients where clinics purchased the donor sperm are not represented, this number varies between countries. These facts might both represent a bias. Therefore, the results should be regarded as tendencies.
Wider implications of the findings: This study contributes to the debate of the importance of providing both anonymous and non-anonymous gamete donors and also for the importance of providing everyone an equal opportunity to establish a family regardless of marital status.
Trial registration number: N/A.

P-080 Sperm chromatin integrity (SCSA analysis) in specific ejaculate fractions can improve semen evaluation under clinical settings

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Study question: Is the standard semen analysis according to World Health Organization (WHO) criteria enough for clinical evaluations prior to fertility interventions?
Summary answer: Fertile semen donors and patients (partners in an infertile couple) with similar WHO-values under evaluation prior to ART-intervention differed in DNA fragmentation index (DFI).
What is known already: Standard semen evaluation is considered insufficient to disclose differences in patients where semen quality is within WHO-acceptable limits, despite proof of subfertility. The SCSA uses flow-cytometry to measure the stability of double-stranded sperm chromatin upon acid exposure. Results are given as%-sperm with denatured DNA, in relation with the total fluorescence measured, which is termed the DNA fragmentation index (DFI). Correlations to fertility after ART are reported (Oleszczuk et al., 2016, Andrology. 10.1011/andr.12153) but yet not widely accepted. Human has a

fractionated ejaculate, with differences in sperm quality, yet samples are still collected/handled in a single tube (bulk ejaculate).
Study design, size, duration: Retrospective. Sperm samples from fertile semen donors ($n = 9$) and patients (partners in an infertile couple with unknown etiology, $n = 8$) under evaluation prior to ART-intervention attending the Reproductive Medicine Centre (RMC), Linköping were frozen for SCSA analysis. Ejaculates were collected as two fractions (F1 = prostate-dominated jets, F2: rest of the ejaculate, seminal vesicle dominated), semen had similar standard (WHO criteria) semen outcome values.
Participants/materials, setting, methods: Consentual fertile semen donors ($n = 9$, 2 ejaculates/person) and patients ($n = 8$, 1 ejaculate/patient) attending clinical setting (RMC, Linköping, Sweden). Ejaculates (donors 2 and patients 1 ejaculate/person) were collected by masturbation in two fractions (F1 & F2). Samples were standard evaluated for volume, sperm concentration (manual counting) and motility (subjective) following WHO-criteria and also for SCSA using flow-cytometry, with outcomes for sperm immaturity (HDS) and DNA fragmentation (DFI). Statistical analysis was performed in R environment (ISBN3-900051-07-0).
Main results and the role of chance: The SCSA showed large variation among individuals and fractions (Table 2), being DNA-fragmentation-index (DFI) per ejaculate (F1+F2) significantly lower in donors than in patients ($P < 0.05$, Kruskal-Wallis rank sum test).

Table 1: Semen characteristics in ejaculate fraction 1 (F1) for the fertile vs patient population (WHO standards 2010, Mean ± SD).

Category	*n*	*n* ejaculates	Volume (mL)	Total sperm number (10^6)	Sperm motility (%)	Round cells	
Total motile	Progressive motile						
Fertile donors	9	18	3.8 ± 1.6	40.8 ± 18.3	67 ± 6.9	60 ± 6.7	No (0–18)
Infertile patients	8	8	1.6 ± 0.3	68 ± 14	71 ± 10	50 ± 3.3	Yes (2/10)

Table 2: DNA fragmentation index (single stranded DNA, DFI, %) and sperm immaturity (HDS, %), Mean ± SEM and ranges in spermatozoa present in the fractions (F1 & F2) of ejaculates from fertile vs patient population; SCSA analysis.

Category	*n*	*n* ejac	Fraction	DFI (%)	DFI range (min–max)	HDS (%)	HDS range (min–max)
			F1	7.1±0.9	3.1–16.1	12.6±2.1	5.5–39.8
Fertile donors	9	18	F2	8.1±1.1	2.7–20.9	12.0±1.9	3.1–36.0
			F1+F2	7.6±1.3[a]	2.7–20.9	12.3±1.4	3.1–39.8
			F1	10.1±2.0	4.3–20.9	11.8±1.5	5.0–17.2
Infertile patients	8	8	F2	13.4±4.0	5.1–39.9	11.6±1.4	6.2–18.2
			F1+F2	11.8±2.2[b]	4.3–39.9	11.7±1.0	5.0–18.2

[a,b]Different superscripts ($p < 0.05$).

Limitations, reasons for caution: Low number of individuals. Sample processing and storage are crucial steps for the accuracy of the SCSA, requiring that $1–2 \times 10^6$ spermatozoa/mL to be extended in TNE buffer and frozen (−20–80C) as soon as possible after sample collection.
Wider implications of the findings: SCSA offers an accurate way to differentiate the status of the sperm DNA. Note that spermatozoa with unstable chromatin remain capable of fertilization. Use of the sperm-rich fraction (F1) eases handling of the ejaculate for diagnostics and, particularly, ART.
Trial registration number: N.A.

P-081 Effects of temperature and concentration on human sperm quality during *in vitro* handling

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Study question: Do the temperature and concentration affect the motility, viability and DNA fragmentation of human sperm during *in vitro* handling?
Summary answer: Viability and DNA integrity of semen or swim-up samples were better preserved at RT versus 37°C. Washed sperm were better preserved at low concentration.
What is known already: Human semen may have a variable degree of sperm DNA fragmentation. Moreover, sperm handling during ART can increase the basal sperm DNA fragmentation and this has been correlated to oxidative stress (OS). As sperm DNA fragmentation and OS may result in poor fertilization, embryo development and even exert long term effects on newborns, it is fundamental to understand the factors that can add a iatrogenic damage to sperm during ART.
Study design, size, duration: Sperm macroscopic and microscopic features were analyzed in 20 patients. Experiment I: semen samples incubated 1h at RT or 37°C. Experiment II: swim up samples incubated 3h at RT or 37°C. Experiment III: washed samples incubated 3h at 37°C at different sperm concentrations (RANGE: 1, 100–75; 2, 50–35; 3, 25–15; 4, $10–5 \times 10^6$).
Samples were analyzed for motility, viability and DNA fragmentation.
Participants/materials, setting, methods: Seventeen normospermic, two oligospermic and one asthenospermic patients were included in the study. Samples were analysed according to WHO (2010) for concentration, motility and kinetics through Sperm Class Analyzer. Viability was evaluated through Live/Dead far red and eosin test; DNA fragmentation was analyzed through the TUNEL assay.
Main results and the role of chance: Experiment I: whole semens samples had a higher viability (time 0, 75.7%; 1h RT, 72.7%; 1h 37°C, 58.6; $P < 0.001$) and lower DNA fragmentation after 1hr incubation at RT than at 37°C (Time 0, 9.4%; 1h RT, 11.7%; 1h 37°C, 21.3%; $P < 0.001$). Experiment II: swim-up samples had a lower DNA fragmentation after 3h at RT than at 37°C (time 0, 9.4%; 3h RT, 13.3%; 3h 37°C, 18.3%; $P < 0.001$). Experiment III: washed sperm samples had a lower DNA fragmentation at low compared to high sperm concentration after 3h of incubation at 37°C (Time 0, 8.3%; Range 1, 17%; Range 2, 13%; Range 3, 10.5%; Range 4, 8%; $P < 0.001$). No significant differences of motility and kinetics were detected in all experiments.
Limitations, reasons for caution: As this is a preliminary study, findings should be confirmed on a higher number of patients.
Wider implications of the findings: As temperature and cell concentration affect the viability and DNA integrity of spermatozoa during *in vitro* handling, it should be avoided to incubate whole semen or swip-up samples for long times at 37°C and at high sperm concentration during routine ART procedures.
Trial registration number: None.

P-082 Abdominal obesity and unbalanced metabolism in men consulting for unexplained subfertility

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Study question: Are male abdominal obesity and metabolic status involved in unexplained subfertility?
Summary answer: BMI and abdominal obesity were increased in subfertile patients, with lower HDL cholesterol levels and higher glycaemia.
What is known already: Overweight and obesity are known to impact male fertility and sperm parameters (Sermondade, 2013). More recently, an impact of metabolic syndrome and abdominal obesity on male reproductive functions has been highlighted (Michalakis, 2013, Metabolism). BMI and abdominal obesity are strongly associated with systemic oxidative stress and low grade inflammation, which could affect the testicular microenvironment and negatively impact sperm production. It could also impact sperm DNA integrity and explain subfertility even if conventional semen parameters are in normal ranges.

Study design, size, duration: Data from subfertile ($n = 97$) and fertile ($n = 98$) men under 45 years recruited in the ALIFERT study were recorded between September 2009 and December 2013. Inclusion criteria excluded patients with current or previous metabolic disease. Subfertile men were partner of subfertile couples. They presented with a primary idiopathic infertility >12 months and had normal sperm characteristics.

Fertile men were included if they had, one child under 2 years spontaneously conceived with a time to pregnancy <12 months.

Participants/materials, setting, methods: Height and waist circumference were measured by the same trained investigator. Weight and body composition were evaluated using the Tanita BC-420MA analyzer. Plasma total cholesterol, high-density lipoprotein (HDL-cholesterol), low-density lipoprotein (LDL-cholestérol), triglycerides and glucose concentrations were measured after a 12-hour fasting period.

Main results and the role of chance: Fertile and subfertile males were similar in term of age (33.2 vs 34.5, $p = 0.061$). BMI (25.9 vs 24.0, $p < 0.001$), waist circumference (91.5 vs 86.3, $p < 0.001$) and abdominal obesity assessed by impedancemetry (6.7 vs 4.1, $p < 0.001$) were increased in subfertile patients. No significant differences of total cholesterol (2.01 vs 2.02, $p = 0.97$), LDL cholesterol (1.28 vs 1.27, $p = 0.89$) and triglycerides (1.26 vs 1.07, $p = 0.077$) levels were observed between fertile and subfertile men. Nevertheless, subfertile patients had lower HDL cholesterol levels (0.49 vs 0.53, $p = 0.011$) and higher glycaemia (4.9 vs 4.3, $p < 0.001$) compared to fertile men.

Limitations, reasons for caution: The definition of unexplained subfertility may be subject to controversy.

Wider implications of the findings: Although subfertile patients presented no alteration of conventional semen parameters, they show higher BMI, higher adiposity and metabolic unbalanced that could explain the difficulty to conceive. Measure of abdominal obesity and assessment of metabolic status should be part of infertility investigations, and treatment should be undertaken before starting ART procedures.

Trial registration number: P071224.

P-083 Land of Fires vs National Park of Cilento (South of Italy): two different enviromental impacts on Sperm DNA Fragmentation

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Study question: The purpose of this study was to evaluate the relationship between areas of residence with different environmental characteristics and the damage extent in spermatic DNA.

Summary answer: Sperm DNA Fragmentation (SDF) differs significantly between (HIP) high environmental impact province and LIP (Low Impact Province).

What is known already: Reproductive health in men is affected by genetic, environmental and lifestyle factors and the adverse effects played by environmental pollution has been evidenced by epidemiological studies. DNA fragmentation levels are a valuable biomarker for male infertility, in particular, the sperm chromatin dispersion test (SCD), giving a SDF value, is considered a reproducible test.

Study design, size, duration: Retrospective study of 175 men underwent to SCD test for infertility, since 2013 to 2015. 500 spermatozoa per DNA test (87,500 total) were analyzed for evaluation of DNA fragmentation by Sperm Chromatin Dispersion method (Halosperm®, Halotech DNA SL).

Participants/materials, setting, methods: 175 healthy non-smoking, non-drinker males, without exposure to environmental factors, selected according to their residence in High (Naples-Caserta province – HIP, $n = 70$), or in Low environmental Impact (Salerno province - LIP, $n = 105$) areas of the Campania region (Southern Italy). The obtained value of SDF were expressed as the percentage of fragmented DNA sperms. The calculation of significance was means of the GraphPad Prism 6.0 software and a value of $P < 0.05$ was considered significant.

Main results and the role of chance: Sperm DNA fragmentation rate significantly increased in High Impact Provinces, well known as "Land of Fires", while is lower in Low Impact Province, the area of National Park of Cilento, (Southern Italy), famous for the origin of Mediterranean diet. A total of 70 samples were analyzed in HIP group (HIP $n = 70$) and 105 samples in LIP group (LIP $n = 105$).

Mean value observed in HIP group was 34 ± 12.3 vs LIP group 27.2 ± 10.9, $P = 0.0003$. The results support the effectiveness of the considered markers in the quantification of DNA damages as well as the relationship between sperm DNA damage extent and the environmental characteristics of the area of residence.

Limitations, reasons for caution: This is an observational study on the relationship between SDF and environmental impact. We don't know the reason of these difference, maybe due to the presence of specific factors. The results support the idea that the relationship between sperm DNA damage and the area of residence can affect male fertility.

Wider implications of the findings: This study confirm that particularly anthropyzed and industrialyzed areas play a key rule in infertility. This study represents a starting point for future analyses to be carried out under the project EcoFoodFertility, which will investigate the role of seminal fluid as a "sensor" of environmental quality in different European areas.

Trial registration number: Not requested. Basic observational retrospective study.

P-084 Sperm DNA fragmentation and mitochondrial membrane potential are better for predicting natural pregnancy than semen analysis

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Study question: Standard semen analysis is not sufficient for natural pregnancy prediction, i.e., in identifying those who are likely to conceive without medical assistance, and those who are not.

Summary answer: Sperm DNA fragmentation and mitochondrial membrane potential - MMP alone or combined may be superior to standard semen parameters in the prediction of natural pregnancy.

What is known already: Semen analysis is more useful in the diagnosis of extreme male factor infertility. An association between sperm DNA fragmentation, MMP and normal fertility potential has been reported previously. Sperm DNA damage has been associated with unsuccessful attempts to conceive. Data on a relationship between DNA sperm fragmentation, measured by TUNEL, and natural conception are scarce and inconsistent. MMP has been shown to be connected wit unsuccessful attempts to achieve assisted conception. An impact of MMP values has never been confirmed on the population of fertile men.

Study design, size, duration: A three-year prospective, cross-sectional was conducted on 51 fertile and 85 infertile couples with no female pathology present. After two year observational period infertile couples were divided regarding the occurrence of conception into subgroup of men who did not conceived - Group IA, and subgroup of men who conceived - Group IB.

Participants/materials, setting, methods: The infertile men were males from infertile couples coming for fertility evaluation with 12–18-month history of unsuccessful attempts to conceive, were observed for additional 6–12 months in terms of achieving a natural pregnancy. Control group were 51 men of currently pregnant women. Sperm DNA and MMP were measured by TUNEL and DiOC&(3) coupled with flow cytometry. The study was carried out at the outpatient infertility clinic, Andrology Unit of the University Medical Centre Ljubljana.

Main results and the role of chance: Twenty-eight of 85 (33%) men from infertile couples conceived naturally after two year observational period. All 28 women delivered after 37 week of pregnancy. The median values of DNA fragmentation and MMP in infertile men were different to those in the fertile controls.

Optimal threshold values of DNA fragmentation and MMP were 22.4% as determined by ROC analysis (AUC 0.69 [95% CI: 0.57–0.81]) and 62.5% (AUC

0.67 [95% CI: (0.55–0.79)]), respectively. The men in the infertile group with values of DNA fragmentation ≤22.4% and with MMP values [3] 62.5% had significantly higher odds for conception (OR 4.92 [95% CI: 1.86–13.02] and OR 4.61 [95% CI: 1.76–12.11], respectively). On contrary all three measures of antioxidative status of seminal plasma did not reach the clinically important discriminative value. Both sperm function tests combined had significant odds for natural conception (OR 6.96 [95% CI: 2.48–19.58]) with probability of 0.64 (64%) for both normal values, and 0.19 (19%) for both abnormal values. Normal combined parameter also influenced time to natural pregnancy achievement.

Limitations, reasons for caution: While this finding is compelling it is not yet conclusive, and should be validated on larger independent sample. Our results demonstrated also some uncertainties as well as false detection rates, which were expected considering the small sample size and still unknown sources of variability connected to unique sperm cell biology.

Wider implications of the findings: Irrespective of the results of standard semen analysis these clearly defined infertile groups of men such as ours will benefit from combined sperm function tests in clinical decision making and hence might avoid unsuccessful attempts to conceive. Such decision making is time and cost effective and has positive effects.

Trial registration number: This study was not a RCT.

P-085 The impact of paternal age on Intra Cytoplasmic Sperm Injection (ICSI) cycle outcome in ART: a retrospective study

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Study question: Does paternal age effect clinical pregnancy, miscarriage and live birth rate in ICSI-cycles?

Summary answer: Miscarriage rates were observed significantly higher in male >40 years old. Live birth rates were significantly lower in >40 years old.

What is known already: There is a tendency for delayed parenthood both for women and men in last decades. It was shown that the miscarriages and chromosomal abnormalities were increasing naturally concieved couples with men over 40 years old. However only seven studies have been focused on paternal age effect on ICSI-cycle outcomes. These studies show discordant findings in terms of effects on embryo quality, pregnancy rate and live birth rate.

Study design, size, duration: We enrolled 1252 couples who have undergone consecutive ICSI with ejeculated spermatozoa between 2010 and 2014. To minimize female age and overian reserve effect women with female age >35 and antral follicle count <5 were excluded. Totally 998 couples were retrospectively analyzed.

Participants/materials, setting, methods: Paternal age were grouped as Group 1 (20–30y, $n = 276$); Group 2 (31–40y, $n = 659$) and Group 3 (41–50y, $n = 63$). Male sperm concentrations, percentage of sperm motility, female body mass index (BMI), antral follicle count were similar between groups. Blastocyst transfer rate were similar in three groups (Group 1, 40.4%; Group 2, 37.1%; and Group 3, 35.2% respectively).

Main results and the role of chance: Clinical pregnancy rate per transfer were comparable in all groups. (Group 1, 31.7%; Group 2, 36.5%; and Group 3, 24.1% respectively). However, miscarriage rates were observed significantly higher in male age group >40 years old compared to others (Group1, 13.2%; Group 2 12.9% and Group 3, 30.7%; $p < 0.001$). Live birth rates per transfer were significantly lower in male age group > 40 years old (Group 1, 20.1%; Group 2, 22.9%; and Group 3, 11.1%, respectively $p < 0.001$). Mean female age in groups were 27.0 ± 3.1, 30.2 ± 3.5 and 32.7 ± 2.6, respectively ($p < 0.001$). Although there was significant difference between groups in terms of female age, we did not clinically take into account due to young female ages in all groups.

Limitations, reasons for caution: Retrospective design of the study and not taking into account of the men's lifestyle factors like smoking could be limitations.

Wider implications of the findings: Analyses of retrospective data demonstrated that miscarriage rate were increased with the paternal age in ICSI-cycles in ART. Live birth rate per transfer were also reduced with the increased paternal age in these patients. Physicians should inform the ART patients about this potential risk.

Trial registration number: NA.

P-086 The effects of smoking, harmful environmental exposure and physical exercise on semen quality

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Study question: The aim of this work is evaluate the influence of life style in semen parameters using a questionnaire that was developed for use in routine infertility consultation.

Summary answer: Men that practice moderate exercise have better semen parameters. For smokers, no differences were found in sperm parameters either they practice exercise or not.

What is known already: Men life style has influence on their fertility. Smoking has impact on sperm motility, sperm count and volume and increases DNA damage, leading to a reduce fertilization capacity. Harmful environment exposure such as heat sources, pesticides and radiation have effect in spermatogenesis. Also, physical exercise appears to be beneficial but only to a certain extent. The moderate practice of exercise can increase the rates of total sperm count, concentration, vitality and motility, and this happens because exercise prevents over and underweight and regulates hormone levels. In men that practice regular and rigorous exercise this parameters have lower rates.

Study design, size, duration: Sperm quality was evaluated in order to see the influence of exercise, smoking and exposure to different environmental conditions in 373 men whom have consulted for couple's infertility during the past 3 years. Subjects are aged between 27 and 46 years old. Semen analysis was performed as part of routine diagnostic testing according to WHO 2010.

Participants/materials, setting, methods: All participants answered an anonymous questionnaire about smoking habits, environmental exposure and quantity of exercise per week. Data obtained was used to classify subjects in 4 groups relating quantity of exercise per week (absent, 1 or 2 times per week and more than 2 times per week), smoking or non smoking and exposure or not to harmful environment factors (such as heat sources). Men with different ages are equally distributed for the 4 groups.

Main results and the role of chance: The percentage of men with normal semen parameter rates, is higher when they practice exercise (mostly moderate exercise) and aren't exposed to harmful environment and don't smoke. Also, non-smokers that practice exercise (moderate or rigorous) and are exposed to harmful environment conditions have better sperm parameters than those that don't practice any exercise.

For smokers, either having exposure to harmful environmental conditions or not, the percentage that presents normal rates of sperm parameters is identical and independent of the physical exercise.

Limitations, reasons for caution: Major limitations are the reduced number of individuals in this study, some questions present in the questionnaire lead sometimes to misunderstandings and other variables like weight, days of abstinence and infections that were not taken into account and may influence the sperm parameters.

Wider implications of the findings: The influence of smoking habits, harmful environment exposure and physical activity is well documented, but information lacks relating the three of them.

This study's results suggest that physical exercise is important in having better sperm parameters, although in smokers, exercise appears to have no influence in sperm parameters.

Trial registration number: NA.

P-087 An investigation of the potential effect of sperm nuclear vacuoles in human sperm on DNA fragmentation using a neutral Comet assay

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Study question: Is there a link between presence of sperm vacuoles and DNA fragmentation?

Summary answer: Lower Motile Sperm Organelle Morphology Examination (MSOME) grading correlates consistently with lower sperm DNA fragmentation.

What is known already: Sperm with fragmented DNA may be morphologically normal and therefore still be selected for fertilization following standard sperm analysis. Assessment of spermatozoa under high magnification provides details about the presence and size of the human sperm vacuoles. Their presence has been already shown to correlate with abnormal chromatin condensation. Studies evaluating the association of sperm morphology and DNA fragmentation have found contradictory results. Currently there is no non-invasive test allowing for determination of spermatozoa DNA damage.

Study design, size, duration: Semen samples were collected from ten partners (mean age 33.6 ± 4.20) of women undergoing IVF treatment at INVICTA Fertility Clinic in August 2015. Standard semen parameters were obtained according to the guidelines of the WHO 2010. The semen samples were prepared using the swim-up method. Men had a normal karyotype, no AZF microdeletions, no CFTR gene mutations. Exclusion criteria were history of varicocele, cryptorchidism, presence of sperm infections, anti-sperm antibodies, smoking and obesity.

Participants/materials, setting, methods: MSOME analysis was performed and spermatozoa were graded into four groups (I-IV) according to Vanderzwalmen's criteria, assigning the best quality spermatozoa, without vacuoles to Grade I. A total of 1869 motile spermatozoa were analyzed by the Neutral Comet assay. Comets were categorized into five classes (0–4) assigning the spermatozoa without DNA fragmentation to Class 0.

Main results and the role of chance: Spermatozoa from MSOME Grade I presented the highest number of spermatozoa without nuclear DNA damage, than Grade II, III, IV groups ($p < 0.05$) as determined with the neutral Comet assay (dsDNA damage).

Grade I spermatozoa presented significantly lower DNA decondensation than Grade II, III, IV ($p < 0.05$).

Our results suggest that even MSOME Grade I spermatozoa shows some DNA fragmentation. In that group about 10% of the spermatozoa presented DNA decondensation, but hardly any (<0.2%) had high degree of DNA fragmentation. Additionally, our results suggest that even MSOME Grade IV spermatozoa normozoospermic patients consist of approximately 32% spermatozoa that have non fragmented nuclear DNA.

Limitations, reasons for caution: The study was limited by relatively small number of normozoospermic patients.

Wider implications of the findings: Our study confirmed the association between nuclear vacuoles and chromatin damage. However, the observation of nuclear vacuoles in the sperm without precise DNA fragmentation investigation is not sufficient for optimal sperm selection for ICSI. New sperm selection methods allowing for low/noninvasive determination of DNA fragmentation are still needed.

Trial registration number: Not applicable.

P-088 Conventional sperm analysis: is it telling us the whole story?

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Study question: Can a comprehensive assessment of sperm quality including gene expression evaluation of protamines reveal male factor functional defects affecting embryo development and ART outcomes?

Summary answer: Altered gene expression ratio of protamine 1 and 2 in human sperm samples is associated to defective embryo development and reduced blastocyst rate.

What is known already: Male infertility impacts 50% of infertile couples. Conventional semen analyses, while helpful, may fail to identify subtle sperm defects, which could influence ART outcomes. A considerable number of studies have reported the utility of evaluating additional sperm parameters to identify these subtle functionality deficiencies. Recent studies have associated aberrant sperm gene expression protamine ratios (mRNA-P1/P2 ratio) and male infertility. Protamines are sperm-specific nuclear proteins essential for packaging the paternal genome in the sperm nucleus. The normal mRNA P1/P2 ratio has been described as 1:1. Variations in this proportion may have implications in sperm function, fertilization and embryo development.

Study design, size, duration: A total of 30 human sperm samples from couples with suspected male factor and at least one previous failed IVF-cycle seeking ART at our center between December 2014 and December 2015 were included in this retrospective study. Comprehensive semen quality analyses including concentration, motility, morphology, apoptosis, sperm DNA fragmentation (SDF) and mRNA-P1/P2 ratio were performed in all samples after 2 days of abstinence. The impact of semen quality parameters on embryo development was explored.

Participants/materials, setting, methods: Conventional semen parameters (concentration, motility and morphology) were analyzed based on WHO criteria (2010). Apoptotic cell levels were evaluated by flow citometry using annexin V-fluorescein isothiocyanate (FITC)/propidium iodide (PI) assay. SDF was assessed using SCSA methodology and mRNA P1/P2 ratio was evaluated by qRT-PCR (Ferticert® test). The impact of semen parameters on embryo development (fertilization rate, blastocyst rate and good morphological quality blastocyst rate) was explored.

Main results and the role of chance: A significant correlation was found between mRNA-P1/P2 ratio and blastocyst rate (Rho Spearman test, $R = 0.680$, $p < 0.05$). Low mRNA-P1/P2 ratio was associated with reduced blastocyst rate and high mRNA-P1/P2 levels were linked to high blastocyst yield. No other sperm parameter was found to significantly correlate with either fertilization or embryo development rates. Normal P1/P2 ratio is established between 0.92–1.08 values; levels below and above this range are considered to be altered.

Samples with low mRNA-P1/P2 ratio showed a blastocyst rate significantly lower when compared to normal mRNA-P1/P2 ratio samples (46.89 ± 8.15 vs 61.69 ± 2.28, $p = 0.034$). High mRNA-P1/P2 samples showed a blastocyst rate significantly higher than controls (93.94 ± 6.06 versus 61.69 ± 2.28 $p = 0.034$).

mRNA-P1/P2 ratio correlated with the percentage of early apoptotic cells (Rho Spearman test, $R = 0.576$, $p < 0.05$). Samples with low mRNA-P1/P2 levels showed lower apoptotic live sperm levels compared to samples with normal mRNA-P1/P2 ratios (15.35 ± 1.11 vs 28.88 ± 11.69, $p = 0.001$). No differences in any of the other semen quality parameters studied were found between the groups.

These results are highlighting the importance of evaluating mRNA-P1/P2 ratio in male factor suspected cases in order to identify functional deficiencies undetected in a conventional spermiogram.

Limitations, reasons for caution: A higher number of samples would be necessary in order to improve the statistical power. Further data regarding clinical outcomes must be included to confirm the predictive value of the analyzed parameters. Female factor controlled cases (ovodonation cycles) would help confirm the conclusions drawn in the present study.

Wider implications of the findings: Gene expression protamine ratio assessment is a reliable and valuable biomarker that offers extra information about sperm functionality. The implementation of this technique as part of the routine spermiogram performed in IVF-centers could be very useful to identify male factor infertility cases and improve diagnosis and ART-treatment of infertile couples.

Trial registration number: None.

P-089 Men born small for gestational age may be at risk of developing male-factor subfertility

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Study question: In males accepted for infertility intervention, are non-optimal birth characteristics associated with a higher risk of male-factor infertility in adulthood?

Summary answer: Men needing infertility treatment are often born with non-optimal birth characteristics suggesting that being born SGA may be a risk factor for infertility in males.

What is known already: Being born with low birth weight, prematurely or SGA increases the risk of developing diseases in adulthood, such as those associated with metabolic syndrome. An association between non-optimal birth characteristics and the reproductive ability in adulthood has been demonstrated in some previous studies, whereas other studies have not been able to find an increased risk of infertility. Men conceiving by ICSI are more often born SGA than men becoming fathers by conventional IVF, suggesting that intrauterine growth restriction may be a risk factor of male factor infertility in adulthood.

Study design, size, duration: Retrospective cohort study of a clinical sample of 1,152 men born in Sweden between 1973 and 1986, partners in couples who following clinical evaluation and diagnosis were accepted for infertility

treatment at Centre of Reproductive Medicine, Linköping University Hospital, 2005–2010. Thirty-three men were excluded due to incomplete investigations. Of the remaining men, 1,067 gave their permission to retrieve information from their medical charts and from the Medical Birth Register (MBR).

Participants/materials, setting, methods: Men were categorized according to infertility type diagnosed by the physician conducting the evaluation of the couple (male, female, combined or unexplained infertility). All information regarding birth characteristics – birth weight, gestational age at birth, prematurity and also perinatal diagnoses – was retrieved from MBR and not self-reported. Men were thus allotted to a reference group (optimal birth characteristics) or an exposed group (non-optimal birth characteristics).

Main results and the role of chance: The cause of infertility was female factor in 27.2%, male factor or combined in 42.5% and unexplained in 30.3%. In the male factor group, 4.5% were born SGA compared with 6.8% in the combined group, 4.1% in the female group and 3.3% in the unexplained group. Despite this tendency, no difference was found when calculating relative risk. When combining the groups of male and combined factor, 11.3% of these men were born with any non-optimal birth characteristic, compared with 10.1% of all men. The prevalence of all of the non-optimal birth characteristics was higher in the male factor and combined infertility groups than in the total material but did not reach statistical significance, partially due to low statistical power.

Limitations, reasons for caution: The major limitation of this study is the relatively small sample size leading to a low absolute number of men in each category of infertility type born with non-optimal birth characteristics. The unexplained infertility group may include individuals where the cause is a male-factor but is yet to be determined.

Wider implications of the findings: Men with male or combined factor infertility are more often born with non-optimal birth characteristics. Male factor is diagnosed entirely by semen analysis using WHO criteria but other parameters could differ between men born with and without optimal birth characteristics. Further studies with larger cohorts will be needed.

Trial registration number: Not applicable.

P-090 Sperm source and early embryonic development

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Study question: Does the source of sperm affect early embryo quality and live birth rate?

Summary answer: Embryo morphology and live birth rate show no differences with respect to sperm source, provided the injected sperm are motile, whether with or without pentoxifylline (PF).

What is known already: There are conflicting data in the literature concerning whether or not the source of sperm (fresh or frozen, ejaculated or surgically retrieved) used in ICSI has an impact on embryo quality and cycle outcome.

Study design, size, duration: This is an observational cohort study of 320 couples undergoing ICSI for severe male factor infertility between 2009 and 2015, of whom 194 had surgical sperm retrieval (SSR) for obstructive and non-obstructive azoospermia, and 126 used ejaculated sperm.

Participants/materials, setting, methods: The following groups of sperm quality were compared: 1) ejaculated motile sperm ($n = 126$); 2) surgically retrieved motile sperm ($n = 83$); 3) surgically retrieved sperm showing motility only after exposure to PF ($n = 76$); 4) surgically retrieved sperm that showed no motility after exposure to PF exposure ($n = 10$). For categorical data, chi-square statistic and for quantitative data, the Student's t tests for unequal variances were used.

Main results and the role of chance: Female age, ovarian reserve, BMI and ovarian response were not significantly different between the groups. There was no significant difference in ICSI fertilisation rate between groups 1 (69%), 2 (61%) and 3 (59%), all of which were significantly higher than group 4 (36%), ($P < 0.01$). Similarly, of the embryos that developed, a higher proportion formed good quality blastocysts when sperm from group 1 (15%), 2 (8%) and 3 (9%) were used, compared to group 4 (0%), ($P < 0.0016$). The live birth rate was 35%, 31%, 29% and 12% for groups 1 to 4th respectively ($P < 0.03$).

Limitations, reasons for caution: This is a cohort study and it is not possible to rule out that more than one variable such as environmental and other female factors could affect the outcome and influence the quality of embryos and live birth rate.

Wider implications of the findings: Contrary to some reports, it appears that the source of sperm has no impact on embryo quality and live birth. Rather it is extremely poor quality sperm that show no motility after PF has a significant impact on embryo quality and live birth rate.

Trial registration number: Not applicable.

P-091 Strict sperm morphology predicts outcome of intrauterine insemination: a prospective observational study

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Study question: This study was intended to evaluated whether value of Kruger strict sperm morphology (SSM) could predict pregnancy after intrauterine insemination (IUI).

Summary answer: The values of Kruger SSM% independently affected IUI pregnancy rates, and cut-off value of 4.0% was selected as a criterion by ROC analysis.

What is known already: SSM values less than 4% were shown to be the best predictor of decreased fertilization and pregnancy rates after IVF. WHO included SSM of greater than 4% as the lower reference value for normal sperm morphology. The association between SSM and IUI outcome has been investigated previously, but study results are conflicting. There is an ongoing debate regarding relevance of SSM for decision making in undergoing IUI.

Study design, size, duration: The study was conducted in prospective observational manner from 2010 to 2015 with 325 non-donor IUI cycles. IRB consent was obtained from each participant.

Participants/materials, setting, methods: The couples attending non-donor IUI were recruited for the study at a single fertility clinic. IUI was performed after spontaneous LH surge or hCG induced ovulation in natural or clomiphene cycle. IUI was prepared using commercial sperm density gradients. Kruger SSM value was calculated from 200 sperms according to 2010 WHO manual and criteria. MedCalc® version 16 was used for statistical analysis.

Main results and the role of chance: The average ages for husband and wife, IUI orders, pre-wash semen volume, total sperm concentration, motility were (mean ± S.D.): 37.8 ± 5.4 & 36.1 ± 4.1 y.o., 2.2 ± 1.7, 2.8 ± 1.4 ml, 196 ± 246 x million/mL, 48.1 ± 17.2%, respectively. The average Kruger SSM value and injected total motile sperm count were: 4.8 ± 3.0%, 23.1 ± 25.0 x million.

There were 29/319(9.1%) positive urine hCG and 26/307(8.4%) positive clinical pregnancy were observed among known outcomes.

The relationship between SSM values and IUI outcomes is summarized as below:

Kruger SSM value	£2.0%	2.0–4.0%	4.0–6.0%	6.0–8.0%	³8.0%
Cycle numbers	55	86	89	42	53
Clinical pregnancies	1	6	7	5	8
Clinical pregnancy rate	1.8%†	7.0%	7.9%	11.9%	15.1%

†: $p = 0.034$ against SSM ≥8.0%.

ROC curve analysis revealed SSM value with 4.0% was selected significance ($p < 0.05$) as a cut-off both for positive urine hCG (sensitivity 62.1%, specificity 62.1%), and for clinical pregnancy (sensitivity 65.4%, specificity 61.2%).

Using wife and husband ages, injected total motile sperms, and Kruger SSM% as independent variables against presence of clinical pregnancy, stepwise logistic regression analysis selected SSM% as the only significant variable ($p = 0.01$) to predict the IUI outcome.

Limitations, reasons for caution: Non-randomized, non-controlled nature of the study. Heterogenous population with various infertility factors, method of ovulation induction, and treatment order.

Wider implications of the findings: There was a declining trend of IUI pregnancy rates with lower Kruger SSM%. Sperm with SSM% lower than 4.0% had significantly lower chance of pregnancy with IUI, and the criteria can be used to counsel infertility couples to accelerate to ART including intracytoplasmic sperm injection to circumvent fertilization failure.

Trial registration number: Not applicable.

P-092 Pregnancy after vas deferens sterilization: surgical repermeabilization or assisted reproduction?

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Study question: Should we opt for surgical repermeabilization or intracytoplasmic sperm injection (ICSI) after a vasectomy?
Summary answer: Repermeabilisation procedure is a good alternative for patients who opt for a more spontaneous solution. Time to pregnancy is longer than in an immediate ICSI traject.
What is known already: About 7.4% of men regret their vasectomy and express a renewed child wish. The choice between surgical vasectomy reversal or ICSI remains difficult for the patient and it's fertility specialist. It remains important for both to be well informed about reversal results and influencing factors of success. Following factors increase probability of conception: surgeons' experience, vasovasostomy instead of epidymovasostomy and female age under 40 years. Peroperative presence of sperm in the vasal fluid and shorter obstructive interval remains unclear in literature. ICSI: intracytoplasmic sperm injection; IVF: in vitro fertilization.
Study design, size, duration: We included 99 male patients from 2006 until 2011 who did a reanastomosis procedure and 64 female patients who did immediately an ICSI treatment where the partner had a vasectomy in the past, this from 2006 until 2011 aswell.
Participants/materials, setting, methods: During a 5 years' period we selected 163 patients. One group of patients (n=99) underwent a reanastomosis procedure after vasectomy. The other group of included patients had a medical history of vasectomy and started immediately with IVF/ICSI (n=64).
Main results and the role of chance: 163 patients, who had a vasectomy in their medical history and have a renewed child wish, from 2006 until 2011 were included. 99 patients underwent a reversal procedure, 64 patients chose immediately for ART. In the overall reversal group the crude cumulative delivery rate (crude CDR) was 49.5%. In this group 45 tried to get pregnant spontaneously, this is the "primary reanastomosis" pathway where the crude CDR was 40.0%. The other 52 patients, which is called the "switchers" pathway, did a reversal procedure and switched to IVF/ICSI, here the crude CDR was 57.4%. Only a few patients opted for insemination (4 cases), where 2 switched again to IVF/ICSI.

The 64 patients who underwent immediately IVF/ICSI had a crude CDR of 43.8% and an expected CDR of 51.6%. This pathway was called the "primary IVF/ICSI" pathway.

The difference in delivery rate of the primary reanastomosis group (40.0%) versus the primary IVF/ICSI group (43.8%) is not significant.

The repermeabilization is a good alternative for patients who don't want to switch to insemination and IVF/ICSI, but they have to wait longer for delivery of a liveborn. If the patient chooses immediately for ART, data shows a good pregnancy rate and faster pregnancy.

The male mean age at vasectomy was 35.5 years, at reanastomosis 44.4 years. The mean obstructive interval was 9.5 years, with maximum 27 years and minimum 1 year. 90% had already children, an average of 2 children, the majority of a previous relation.
Limitations, reasons for caution: The study population is rather small (n= 163).
Study funding/competing interest(s): No conflict of interest are declared. No funding was used for this study.
Wider implications of the findings: Better counseling possibilities for couples with a vasectomy in history. It can help physicians and patients to make an optimal decision in case of a renewed childwish in these patients. If time to pregnancy is important, we suggest to chose immediately for IVF/ICSI treatment.
Trial registration number: Not necessary for this type of study.

P-093 Human papilloma virus sperm infection and its correlation with *in vitro* fertilization

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Study question: To evaluate the reproductive outcome of infertile couple undergoing assisted reproduction technique with or without HPV (Human Papilloma Virus) semen infection.
Summary answer: We found a correlation between the presence of HPV at sperm level and the reduction in assisted pregnancy rate.
What is known already: HPV infection is one of the most common sexually transmitted disease. It has been detected in multiple anatomic sites, including the cervix, anus, penis, and some areas of unprotected genital skin, such as vulva and scrotum.

In males, HPV DNA has been found in external genitalia, in urethra, in the deferent ducts, epididymis and testis, and seminal fluid too.

Some authors report a reduction of pregnancy in *in vitro* fertilization in presence of HPV cervical infection compared with no infection, as reported by some authors, but very few works associates human HPV sperm infection to *in vitro* fertilization results.
Study design, size, duration: A total of 156 men were enrolled between January and December 2015. Their semen were tested for the presence of HPV in semen.

82 of them underwent to *in vitro* fertilization and they were included in our retrospective study. Couples were divided into two groups based on the presence or absence of HPV semen infection. The presence of HPV was related to clinical pregnancy rate.
Participants/materials, setting, methods: Study was conducted in the Department of Obstetrics, Gynecology and Physiopathology of Human Reproduction, Hospital S. Luca, Vallo della Lucania, SA, Italy. No patient enrolled resulted positive for HIV, HCV, HBV and treponema pallidum. No patient showed azoospermia, leukospermia, anti sperm antibodies and varicocele.

Women showed normal BMI and idiopathic infertility.

Sperm parameters were evaluated according to the World Health Organization manual, and the presence of HPV DNA researched by the use of HPV assay and a highly sensitive nested polymerase chain reaction assay.
Main results and the role of chance: 23 of the total semen samples were HPV positive.

Median value of sperm concentration was 40.55 millions/ml and 49.44 millions/ml in HPV negative and HPV positive group, respectively ($P > 0.05$).

HPV non infected and HPV infected semen samples had similar morphology (medians 6.39% vs 5.69%; $P > 0.05$), but different progressive motility (median 17.54% vs 7.69%; $P < 0.05$).

We evaluated the amount of DNA fragmented sperm and found an a similar dfi (DNA fragmented index) in the two groups (30.75% vs 27.99%; $P > 0.05$).

15 out of 64 couples without HPV semen infection had successful I C S I (23.4%), and 2 out of 18 couple (11.1%) in the infected group had successful ICSI ($P < 0.05$)
Limitations, reasons for caution: Differences between data were determined by two-tail to student's test after acceptance of normal distribution with the Kolmogorov-Smivov test.

P value (two sided) of less than 0.05 were considered to statistically significant. Our results are supported by a concise study design but with a small sample size.
Wider implications of the findings: In literature there are few studies that demonstrates a correlation between HPV sperm infection and reduction of pregnancy rate. If our results are confirmed they could change the clinical approach to HPV infected couples.
Trial registration number: None.

P-094 Association of serum vitamin D with sex hormones and semen quality

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Study question: The objective of this study was to investigate the association between serum 25(OH) vitamin D with semen quality and hormones in Iranian subfertile men.
Summary answer: High incidence of 25(OH) D deficiency and insufficiency were observed in our participants. It looks that it is correlated to sperm motility in AOT men.

What is known already: Vitamin D deficiency is one of the most common health problem in worldwide and Iran. Previous studies have shown that vitamin D is involved various adverse events including hypertension, cardiovascular disease, stroke, diabetes and cancer.

The issue of whether vitamin D levels are effective factor for male reproductive biology is suspected and conflict data exists on the potential association between serum vitamin D and semen quality and sex hormones.

Study design, size, duration: This cross - sectional study, during March-September 2014, was conducted on 278 men (ranged 20–50 yr. old) referring to Royan infertility clinic (Tehran, Iran), consisted of 186 normospermic and 92 AOT (Astheno-, Oligo- and Terato spermic) groups according to WHO criteria. Blood and semen samples were obtained for assessment of sex hormones, 25-hydroxyvitamin D, parathyroid hormone, calcium and semen parameters. Vitamin D levels were classified as deficiency (≤20ng/ml), insufficiency (21–29ng/ml) and normal (≥30ng/ml).

Participants/materials, setting, methods: Semen analysis was examined after 2–5 days of sexual abstinence based on WHO-recommended methods by CASA system (computer-assisted sperm analysis) measurement of serum 25-hydroxyvitamin D, Parathyroid hormone (PTH) and Calcium and sex hormones were done by ELIZA.

Main results and the role of chance: Vitamin D deficiency, insufficiency and normal levels were observed in 58.6%, 29.5% and 11.9% of participants respectively. Serum 25 (OH) vitamin D was inversely correlated with PTH ($P < 0.045$). In normospermic men, serum 25 (OH) D levels and categorized were not correlated with semen parameters and reproductive hormones. But sperm motility showed a positive correlation with 25 (OH) D categorized in AOT men ($r = 0.131$, $p = 0.028$).

Limitations, reasons for caution: No limitation.

Wider implications of the findings: Last cross-sectional studies have found the association of serum 25(OH) D levels and semen parameters in young healthy men, but the results were quite discordant.

Trial registration number: No.

P-095 Next generation sequencing reveals a novel mutation in the XY-linker region of phospholipase C zeta (PLCζ), resulting in truncated protein and oocyte activation deficiency

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Study question: Is the novel mutation located in the XY-linker region of PLCζ the possible cause of repeated low fertilization rates or total fertilization failure (TFF) in a patient?

Summary answer: The novel mutation in PLCζ at position 322 of the amino acid sequence results in a significantly truncated and non-functional form of PLCζ.

What is known already: PLCζ is a sperm-specific protein responsible for oocyte activation, and abnormalities in the expression, structure and localization of PLCζ have been causally linked to oocyte activation deficiency (OAD) and male factor infertility. While the precise etiology of OAD remains inconclusive, three rare genetic anomalies have already been described for PLCζ in patients experiencing OAD (His398Pro, His233Leu and Ile489Phe).

Study design, size, duration: A case study of a male patient who experienced repeated low fertilization outcome or TFF throughout a series of four failed intracytoplasmic sperm injection (ICSI) cycles, and to evaluate the potential role played by PLCζ deficiency.

Participants/materials, setting, methods: The patient's sperm sample was first subjected to the mouse oocyte activation test (MOAT) and then analyzed by exome sequencing. A predicted three-dimensional model of the mutant PLCζ was designed using Modeller and PyMOL. The ability of the mutation to induce calcium oscillations was evaluated by injecting mutant PLCζ cRNA into mouse oocytes, and comparing the resultant calcium release profile to that induced by control wild type PLCζ cRNA.

Main results and the role of chance: Five out of 26 mouse oocytes (19%) were activated following MOAT, thus categorizing our patient as a low MOAT subject in which a sperm-related deficiency was the most likely cause of infertility. Exome sequencing revealed a change of amino acid from a Lysine (K) to a stop codon at position 322 (K322STOP). The location of mutation is at the region of the PLCζ gene which encodes the XY-linker. Next generation sequencing (NGS) subsequently confirmed the presence of the mutation in both buccal and sperm samples from the patient. NGS also identified the presence of a mixed population of normal (53%) and mutant (47%) sperm in the patient's semen. A predicted three-dimensional model of PLCζ K322STOP revealed a severely compromised protein, in which 286 amino acids (47%) were missing in contrast to wild type PLCζ. The absence of amino acids resulted in the lacking of several critical catalytic residues and the entire Y and C2 functional domains. Four out of six mouse oocytes injected with wild type PLCζ cRNA successfully induced calcium oscillations in a form which were characteristic of normal fertilization, whereas injections of PLCζ K322STOP cRNA failed to cause calcium release (0/7 mouse oocytes).

Limitations, reasons for caution: Since the three-dimensional structure of wild type PLCζ has not yet been determined, our present model of PLCζ structure was created by reference to the structures of other PLCs. Consequently, it is only possible to speculate upon the potential effect of PLCζ K322STOP on PLCζ structure and function.

Wider implications of the findings: Our data describes the most deleterious PLCζ mutation discovered thus far, which results in a translated protein that is truncated by 47% of the wild type protein and therefore is incapable of causing calcium release when injected into mouse oocytes.

Trial registration number: NA.

POSTER VIEWING SESSION

EARLY PREGNANCY

P-096 Genotyping analysis of protein S-Tokushima and the involvement of protein S antigen and activity in patients with recurrent pregnancy loss

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Study question: To determine whether a protein S (PS) deficiency and PS-Tokushima can influence a subsequent pregnancy outcome in patients with recurrent pregnancy loss (RPL).

Summary answer: There was no association between PS-Tokushima and RPL, and a PS deficiency or low PS activity was not a reliable clinical predictor of subsequent miscarriage.

What is known already: A meta-analysis revealed that PS deficiency is associated with recurrent fetal loss and late non-recurrent fetal loss but not with recurrent early miscarriage.

The PS-Tokushima missense mutation was reported to serve as a genetic risk factor for DVT in the Japanese population. A previous cross-sectional study showed no increase in the prevalence of PS-Tokushima in patients with recurrent early pregnancy loss or in patients with IUFD and/or fetal growth restriction. There has been limited number of prospective studies examining the pregnancy outcome in patients with both a PS deficiency and recurrent RPL.

Study design, size, duration: The frequencies of PS-Tokushima were compared between 355 patients and 101 controls. The total PS antigen, activity, and specific activity were compared between the 210 patients and 101 controls since only 210 patients were available to provide plasma samples.

All patients were seen between September 2008 and July 2014, and subsequent pregnancies of all patients were followed up until December 14, 2014.

Participants/materials, setting, methods: The study group consisted of 355 Japanese women with two or more consecutive pregnancy losses. Control subjects consisted of 101 women with at least one child and no history of infertility or miscarriage.

To confirm the genotype, purified templates were sequenced with a BigDye Terminator v3.1 Cycle Sequencing kit. The PS antigen, activity, and specific activity (total PS activity/total PS antigen) were determined with a total PS-assay system.

Main results and the role of chance: Nine of 355 (2.5%) patients and one of the 101 (1.0%) controls were positive for PS-Tokushima. There was no significant difference in the prevalence of the mutation.

There were no differences in levels of PS antigen, PS activity and PS-specific activity between patients and controls.

All 8 patients with PS Tokushima gave live birth without heparin and two patients had live births with aspirin alone. One patient with PS Tokushima and a history of IUFD miscarried again in spite of heparin use. There was no significant difference in subsequent live birth rates between patients with low or normal PS-specific activity/PS activity without heparin prophylaxis after excluding miscarriages caused by an abnormal embryonic karyotype using multivariate logistic regression analysis (OR, 1.84; 95% CI, 0.59-5.75/OR, 3.40; 95% CI, 0.96–12.07).

Limitations, reasons for caution: We were able to measure total PS antigen and activity in only 210 patients because of the lack of plasma samples. Another limitation was that we examined only 20 patients with IUFD.

Wider implications of the findings: The PS Tokushima mutation and low PS activity were not risk factors for early RPL. Therefore, we propose that testing for PS antigen and/or activity is not needed, as it is without clinical benefit and constitutes an unnecessary expense.

Trial registration number: N/A.

P-097 Efficacy of intrauterine injection of granulocyte colony stimulating factor (G-CSF) on treatment of unexplained recurrent miscarriage

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Study question: Does G-CSF improve pregnancy outcomes in patients with recurrent miscarriage?

Summary answer: we couldn't suggest intrauterine injection of G-CSF for improvement of clinical pregnancy rate and reduce of abortion among patients with unexplained RM.

What is known already: GCS-F (Granulocyte Colony-stimulating Factor) is an important cytokine with critical role in embryo implantation and pregnancy. The previous studies showed that that transvaginal endometrial perfusion with G-CSF might be helpful for improvement of implantation rate among patients with thin endometrium and repeated implantation failure (RIF).

Study design, size, duration: In present randomized clinical trial, during a 12-month period, a total of 68 patients were randomly allocated into two study groups including intrauterine G-CSF (300µg, Filgrastim, Switzerland) injection and control group (no G-CSF injection). All patients were in I/O (Ovulation Induction) cycle. In G-CSF group, intrauterine injection of G-CSF was done twice in cycle. All enrolled patients were under 40 years old and had at least two times unexplained pregnancy loss.

Participants/materials, setting, methods: Pregnancy was evaluated by titer of βhCG, presence of gestational sac (implantation) was assessed by vaginal ultrasonography and finally clinical pregnancy was confirmed by detection of fetal heart rate (FHR).

Main results and the role of chance: Eighteen out of 68 patients were excluded from the final analysis due to different reasons. No significant difference were observed between two study groups when we compared the rate of chemical pregnancy (26.1% vs. 29.6%, $P = 1.000$), implantation (26.1% vs. 22.2%, Fisher's exact test $P = 0.673$), clinical pregnancy (17.4% vs. 11.1%, Fisher's exact test P: 0.657) and abortion (8.7% vs. 18.5% Fisher's exact test $P = 0.921$).

Limitations, reasons for caution: In previous studies, intrauterine injection of G-CSF was not evaluated in recurrent miscarriage patients. So we can not compare our results with them.

Wider implications of the findings: In previous studies, intrauterine injection of G-CSF was not evaluated in recurrent miscarriage patients. So we can not compare our results with them.

Trial registration number: IRCT2013012211430N3.

P-098 TSHR as a new susceptibility locus: The first genome-wide association study for obstetric antiphospholipid syndrome

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Study question: Is genetic susceptibility associated with obstetric antiphospholipid syndrome (APS)?

Summary answer: Our findings demonstrate that a *TSHR* risk allele-homozygous genotype and an *HLA-DQB1*05:01* allele-negative genotype can indicate a susceptiblity for obstetric APS in the Japanese population.

What is known already: APS is the most important treatable cause of recurrent pregnancy loss (RPL). However, the live birth rate is only about 70–80% with the current treatment. Recent genome-wide association studies (GWAS) of systemic lupus erythematosus (SLE) have shown that a number of molecules may be involved in the pathophysiology of B cell/T cell responses and the NF-kB signaling pathway. Since APS is a lupus-related disease, genetic susceptibility has been speculated to be associated with obstetric APS. This is the first GWAS for obstetric APS focusing on RPL.

Study design, size, duration: A GWAS was performed to compare 115 Japanese patients with obstetric APS diagnosed according to criteria of the International Congress on APS and 419 healthy individuals. Samples were collected between November 2009 and March 2014.

Participants/materials, setting, methods: Among the 155 patients recruited in this study, we decided to include 115 patients with strongly positive for lupus anticoagulant. 600,307 SNPs were genotyped and allele or genotype frequencies were compared. Imputation analyses were also performed for the candidate regions detected by the GWAS.

Main results and the role of chance: One SNP (rs2288493) located on the 3'-UTR of *TSHR* showed a significant APS association ($P = 7.85E-08$, OR = 6.18) under a recessive model even after applying a Bonferroni correction by the number of analyzed SNPs. A total of 56 SNPs from 11 distinct genomic regions, *TSHR*, *NGF*, *C1D*, *CDH18*, *SYCP2L*, *HLA-DRA*, *HCRTR2*, *GATA3*, *FRMD4A*, *PTPRO* and *MRPS23,* showed evidence suggestive of an association ($P < 5E-06$). In addition, analysis of *HLA* alleles revealed that the *HLA-DQB1*05:01* allele was protective with a significant association ($P = 0.0037$, OR = 0.28).

Limitations, reasons for caution: Sample size is relatively small. Further study will help to confirm the biological significance of the genes implicated in APS, and will ultimately result in specific treatment to improve the pregnancy outcome of the affected patients.

Wider implications of the findings: In the present study, not only *TSHR* but also other genes suggestive of APS have supplied us with clues to account for the unexplained mechanisms in early miscarriage, fetal loss, preeclampsia and many features of this disease.

Trial registration number: N/A.

P-099 Local injection of diluent vasopressin followed by suction curettage as initial treatment of cervical ectopic pregnancy

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Study question: Is injection of diluent vasopressin to the uterine cervix and myometrium followed by suction curettage safe and effective as treatment of cervical ectopic pregnancy?
Summary answer: The procedure is a safe and effective because it is easy to perform, is not associated with any critical complications, and possibly minimizes blood loss.
What is known already: Conservative treatment options for cervical pregnancy are local and general administrations of methotrexate, dilatation and curettage with or without local injection of potassium chloride or methotrexate, uterine artery embolization, and their combination. However, uncontrolled massive bleeding during the procedures requires hysterectomy. Local injection of diluent vasopressin to the myometrium surrounding the uterine fibroids is a well-established technique for reducing blood loss during myomectomy and hysterectomy. Vasopressin has not only a vasoconstrictive effect but also a smooth muscle contractive activity.
Study design, size, duration: This is a retrospective review of 13 consecutive first-trimester cervical pregnancies from 2007 to 2015. We conducted a chart review of data on total blood loss, side effects during the procedure, and duration until conversion of serum human chorionic gonadotropin (hCG) assay results to negative.
Participants/materials, setting, methods: We injected diluent vasopressin 10 to 100 times to the cervix surrounding a gestational sac and/or myometrium of the lower segment under transvaginal ultrasonographic guidance in women who were affected with cervical pregnancy. For cases where the fetal heart beat remained active after the injection, we injected additional vasopressin into the gestational sac. After confirming that the fetal heartbeat had stopped, we aspirated the chorionic villi, including the gestational sac, at 60 mm Hg.
Main results and the role of chance: The mean age and serum hCG level of the patients before the procedure were 31.4 ± 7.2 years and 16,929 ± 20,869 mIU/mL, respectively. The mean duration between the procedure and the conversion of serum hCG assay results to negative was 57.4 ± 22.2 days. We observed spontaneous evacuation of the villi containing a gestational sac to the external os after the vasopressin injection in 4 of the 13 patients. The procedural duration was shorter than 15 minutes, and the whole blood loss volume during the procedure was less than 100 mL in all the cases. The vasopressin injection and subsequent suction curettage had no adverse effects during the procedure. Additional methotrexate administration was needed in one patient because of persistent trophoblastic disease after the curettage. None of the patients required blood transfusion and additional uterine artery embolization or hysterectomy.
Limitations, reasons for caution: This observational study conducted a retrospective chart review and included a relatively small sample size.
Wider implications of the findings: Local injection of diluent vasopressin followed by suction curettage is a dominant treatment option for cervical pregnancy in patients who wish to preserve future fecundity. Spontaneous evacuation of the gestational sac is also expected, with minimal blood loss.
Trial registration number: Not applicable.

P-100 The association between first trimester uterine artery Doppler velocimetry indices and adverse perinatal outcomes in IVF cycles

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Study question: This study aimed at evaluating if first trimester uterine artery doppler velocimetry values may predict adverse perinatal outcomes in patients with IVF
Summary answer: The first trimester uterine artery doppler velocimetry indices including RI and S/D values are good parameters to predict adverse perinatal outcomes in IVF pregnancies
What is known already: Pregnancies following assisted reproductive techniques (ART) are at increased risk of maternal and perinatal complications. The fetal well being is generally evaluated by the biophysical profile (BPP) and the non-stress test (NST) and doppler velocimetry evaluation. Doppler velocimetry studies may evaluate the status of the pregnant women by indirectly examining uteroplacental perfusion adequacy with uterine artery doppler velocimetry indices. Previous studies reported that doppler studies in the first trimester of pregnancy may predict the adverse perinatal outcomes in spontaneous pregnancies.
Study design, size, duration: This prospective cross sectional study was designed at Department of Reproductive Endocrinology from May 2013 to June 2014. A total of sixty two infertile women who underwent IVF procedure and became singleton pregnancy (group 1 = study) and a fifty three healthy women were selected randomly among the pregnant women who attended to routine antenatal follow-up program (group 2 = control) were included to the study.
Participants/materials, setting, methods: After a routine fetal scan the cases had transvaginal doppler velocimetry from bilateral uterine arteries. The doppler velocimetry measured were; right and left side uterine artery (RUA/LUA) systole-diastole ratio (SD), pulsatility index (PI) and resistance index (RI). Adverse perinatal outcomes (delivery type, preterm birth (delivery occurred at gestational ages between 22 and 36 weeks), low birth weight, low APGAR score and neonatal intensive care unit (NICU) requirement recorded and compared with the doppler velocimetry.
Main results and the role of chance: There were no statically significantly difference between groups in terms of; BMI, week of doppler measurement, LUAPI and RUAPI levels ($p > 0.05$). Gestational age at birth, first minute APGAR scores, NICU requirement ratios, birth weight, preterm birth ratios, abortion ratios, delivery type, LUASD, LUARI, RUASD, RUARI levels were statically significant difference between groups ($p < 0.05$). According to the ROC curve analysis LUASD, RUASD and RUARI were predictive for preterm birth and low birth weight and LUARI for NICU. Correlation analysis showed that there was a positive correlation between LUASD, LUARI, RUASD, RUARI levels and preterm birth ratios, NICU requirement ratios in the study group. And also, birth weight showed a negative correlation in terms of LUASD, LUARI, RUASD and RUARI levels in the study group. Caesarean deliveries were higher in the study group. The incidence of preterm birth was significantly higher in IVF pregnancies ($p < 0.05$).
Limitations, reasons for caution: The limitation of this study is small sample size. However, this sample size was sufficient to show statistically significant differences in uterine artery doppler velocimetry parameters between the groups. No other sonographic factors such as uterine artery doppler velocimetry, biophysic profile, etc. was performed to evaluate fetal well being before delivery.
Wider implications of the findings: The first trimester uterine artery doppler velocimetry indices including RI, S/D and PI values are good parameters to predict adverse perinatal outcomes. For this, IVF pregnancies with bad uterine artery doppler result in the first trimester should be examined closely to predict adverse perinatal outcomes.
Trial registration number: None.

P-101 Area under the curve of estradiol monitorisation: a novel approach to evaluate detrimental effect of estrogen exposure on implantation along the COH

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Study question: To assess the utility of area under the curve of estradiol monitorisation during ART cycles to predict failure of implantation and clinical pregnancy.

Summary answer: Calculation of the area under the estradiol monitorisation curve during ART cycles may be used to predict failure of implantation and clinical pregnancy.
What is known already: Low levels of exogenous estradiol maintained the responsive endometrium for a long period of time, however high doses of estradiol lead to development of refractory endometrium. Estradiol in a narrow range is a key factor for the duration of the window of implantation in uterine receptivity in mice. There is a possibility of receptivity window manipulation by different doses of estradiol.
Study design, size, duration: Prospective data analyses to assess the Area under the curve of estradiol monitorisation (AUCEM) during ART cycles and compare between the age and BMI matched groups of infertile women with (n = 109) and without (n = 173) successful embryo implantation.
Participants/materials, setting, methods: After approval of the Hospital Ethics Committee and consents from all participants, study was conducted from January 2014 to December 2014 in the IVF/ICSI unit of Zeynep Kamil Women and Children's health Training and Research hospital. A total of 282 women who underwent ART were prospectively screened and AUCEM was calculated for each participant. Patients were divided into two groups with (n = 109) and without (n = 173) successful implantation.
Main results and the role of chance: Comparison of groups with and without positive clinical implantation showed a significant difference between the groups in terms of area under the curve of estradiol monitorisation, estradiol per day and the endometrial thickness at trigger day (P = 0.05). Additionally, comparison of groups with and without positive clinical pregnancy showed a significant difference between the groups in terms of area under the curve of estradiol monitorisation, estradiol per day and the endometrial thickness at trigger day (P = 0.05).
Limitations, reasons for caution: A larger sample size is required to generalize the results. Our study lack the comparison between the previously reported tools like endometrial receptivity array and the AUCEM and we have no data with regard to predictive value of AUCEM for live births.
Wider implications of the findings: According to our results it may be considered to postpone embryo transfer in cases with high AUCEM and prefer freezing all embryos.
Trial registration number: Trial registration was not needed.

P-102 Optimism in live birth rates of unicornuate uterus patients

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Study question: To analyse the effect of a unicornuate uterus anomaly on the reproductive outcomes of IVF treatment.
Summary answer: Pregnancies in patients with unicornuate uteruses are high-risk pregnancies with poor perinatal and obstetric outcomes that require intensive obstetric observation and care, and mandatory SET.
What is known already: Unicornuate uterus is a relatively uncommon congenital uterine anomaly, classified as a type II anomaly, with a predicted incidence of 1:4000 in the general population. This anomaly has been reported to have poor reproductive outcomes from spontaneous pregnancies, i.e., increased extra-uterine implantation, placental complications, first and second trimester miscarriage, fetal mal-presentation, intrauterine growth retardation, intrauterine fetal demise, and preterm birth.
Study design, size, duration: In this retrospective observational study, the files of patients who presented for first consultations at one single ART center, during the period from January 2012 to December 2015, were screened for the diagnosis of unicornuate uterus. The identified files were analysed in respect of medical/fertility history, diagnostics, and the reproductive outcomes of ART treatments received.
Participants/materials, setting, methods: During the study period, 5704 first consultations were performed and 48 diagnoses (0.84%) of unicornuate uterus were confirmed.
Main results and the role of chance: The mean age of the diagnosed patients was 31.0 years, with a mean antral follicle count of 20.3. Of the 48 patients diagnosed, 37 underwent ART treatment, including fresh (n = 21) and frozen ET (n = 39). The overall pregnancy and clinical pregnancy rates were, 62.0% and 50.0% respectively. The clinical pregnancies included one (3.2%) ectopic pregnancy. Twenty pregnancies have progressed beyond week 14 of gestation, and of these 15 have delivered. The 15 live births were delivered at a mean gestational age of 36.5 weeks and birthweight of 271.7 g, with 66.7% premature and 26.7% low birth weight (LBW).
Limitations, reasons for caution: Although the study includes a large number of ART treatments for a low incidence intrauterine anomaly such as unicornuate uterus, the study is retrospective and has inherent limitations and biases.
Wider implications of the findings: The study highlights the high pregnancy loss and premature delivery rate associated with unicornuate uteruses and, therefore, the mandatory need for SET, but also that more than 60% of clinical pregnancies result in a live delivery.
Trial registration number: N/A.

P-103 HLA-E was involved in the effect of progesterone on the ADAM19 expression in JEG-3 cells

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Study question: To investigate if HLA-E, one of the MHC-Ib molecules expressed in trophoblast cells plays a role in the regulation of ADAM19 by progesterone.
Summary answer: The silence of HLA-E expression decreased the expression level of ADAM19 and diminished the up-regulatory effect of progesterone on ADAM19 in trophoblast cell line JEG-3.
What is known already: ADAM19 is highly expressed in human placentas and suggested to be involved in the key processes of trophoblast invasion and degradation of extracellular matrix during early pregnancy, but the regulation of ADAM19 in trophoblasts has been poorly understood. HLA-E, one of the MHC-Ib molecules, contributed to the establishment of an immune tolerance at maternal-fetal interface during pregnancy. In our previous study, both ADAM19 and HLA-E were up-regulated following progesterone treatment in trophoblast cell line JEG3. We performed this study to determine if HLA-E plays a role in the regulation of invasive-related gene ADAM19 in trophoblast cells.
Study design, size, duration: This control study was carried out over a one year period. The JEG-3 cells were divided into 5 groups: the blank control (group 1), progesterone treatment (group 2), transfection of lentivirus carrying siRNA targeting HLA-E before progesterone treatment (group 3), transfection of lentivirus carrying siRNA targeting HLA-E (group 4) and transfection of the negative control siRNA (group 5). Independent experiments were repeated three times with triplicates for each treatment.
Participants/materials, setting, methods: The JEG-3 cells were obtained from The JEG-3 cells (Cell Resource Center, IBMS, CAMS/PUMC). The expression silence of HLA-E in JEG-3 cells was induced through transfection with the lentivirus carrying siRNA targeting HLA-E to examine its effect on the expression of ADAM19. In 5 groups cells were collected 48 h after different treatments. The mRNA and protein levels of ADAM19 in JEG-3 cells were detected by real-time quantitative PCR and Western blot assay, respectively.
Main results and the role of chance: In this study, the expression level of ADAM19 mRNA in JEG-3 was up-regulated following treatment of progesterone (group 2) while down-regulated after transfected with lentivirus carrying siRNA targeting HLA-E (group 4), both with significant difference compared to the control groups. In group 3, the HLA-E in JEG-3 was silenced by transfection of siRNA targeting HLA-E before progesterone treatment. Data showed that there was no significant difference in ADAM 19 mRNA expression level compared with the controls, indicating that the up-regulatory effect of progesterone on ADAM19 mRNA expression in JEG-3 was abolished by HLA-E silence. The protein levels detected in group 2, 3 and 4 showed similar changing tendency as observed in mRNA levels, when compared with the controls.
Limitations, reasons for caution: JEG-3 cell line, a common extravillous trophoblast cell (EVT) model, was used in this study to investigate the regulation of ADAM19. Further studies are necessary to determine if HLA-E has the same effect on ADAM19 expression in trophoblast cells from early gestational placenta.

Wider implications of the findings: HLA-E, although not fully understood, contributes to the maintenance of pregnancy. We found in this study that HLA-E was involved in the regulation of ADAM19 in EVT model JEG-3, which may provide a new clue to investigate the regulation of invasive ability of trophoblasts at maternal-fetal interface.
Trial registration number: None.

P-104 Expression of hypoxia inducible factor and vascularization status was altered in peri-implantation endometrium of women with recurrent miscarriage

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Study question: To compare the expression of hypoxia inducible factor 1α (HIF1α) and micro blood vessels in peri-implantation endometrium of women with recurrent miscarriage and fertile controls.
Summary answer: The aberrant expressions of HIF1α and blood vessels in peri-implantation endometrium suggested altered hypoxia and vascularization status may account for endometrial contribution to recurrent miscarriage.
What is known already: In the unexplained recurrent miscarriage cases, embryo aneuploidy occurred in just less than 50% cases, indicating that subtle endometrial factors may account for the remaining cases. The vascularization and oxygen status of endometrium plays an important role in the success of pregnancy and it is now well accepted that HIF1α is the main mediator of the regulation of oxygen homeostasis and many other cellular processes in normal cells.
Study design, size, duration: 24 women diagnosed as recurrent miscarriage and 36 women of proven fertility were recruited from two university hospitals from November 2014 to October 2015. Endometrial biopsy samples were obtained precisely 7 days after luteinization hormone surge in a natural cycle.
Participants/materials, setting, methods: Immunohistochemistry was used to determine expression of HIF1α and micro blood vessels identified by von Willebrand factor (vWF) in endometrium. A semi-quantitative analysis was performed by using *H*-score analysis of staining intensity for HIF1α in the luminal epithelium, glandular epithelium and stroma, separately. The number, diameter and volume of vWF-positive endometrial micro vessels were counted by ImageJ software.
Main results and the role of chance: A significantly higher endometrial expression of HIF1α was observed in luminal epithelium ($P = 0.007$) and stroma ($P = 0.039$) in women with recurrent miscarriage compared to fertile controls. There was no significant difference in HIF1α expression in glandular epithelium between both groups. An increased number of micro blood vessels ($P = 0.008$) as well as volume of blood vessels ($P = 0.008$) was found in women with recurrent miscarriage. The mean diameter of blood vessels did not differ significantly between two groups.
Limitations, reasons for caution: Immunohistochemistry and *H*-score analysis for staining intensity are semi-quantitative methods to determine the amount of protein expression. The study could potentially be strengthened by the micro dissection of endometrium into different compartments and protein levels may be measured by quantitative ELISA kit.
Wider implications of the findings: The significantly altered expression of HIF1α as well as increased number and volume of micro blood vessels in the peri-implantation endometrium provides a molecular explanation for the endometrial factors contributing to recurrent miscarriage.
Trial registration number: NA.

P-105 Flow cytometric evaluation of epithelial b3-Integrin expression in endometrium of recurrent miscarriage patients and correlation with endometrial receptivity array

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Study question: Can a simple cost effective flow cytometric technique be used to screen candidates into those who are receptive and those requiring the more expensive endometrial receptivity array (ERA) for assessment.
Summary answer: Percentage epithelial β3 integrin expression correlates reasonably well to the gold standard 238 gene profile array of the Igenomics ERA test.
What is known already: Many techniques now exist to assess the receptivity status of female endometrial tissue and range from fully validated molecular techniques such as the ERA (or endometrial receptivity array) which is a genetic test to immunohistochemical techniques on fixed and stained tissue slides assessing various markers such as Cyclin E, p27 and β3 integrin. All are characterised by relatively high cost and considerable time to results. Flow cytometry is generally not employed for this type of evaluation but correctly set up allows for an almost instantaneous low-cost assessment of the material.
Study design, size, duration: The receptivity status of 23 IVF patients was assessed using both the ERA and FCM epithelial β3 integrin evaluation designed and applied in our Clinic. We set an initial cut off value of 60% of epithelial cells expressing β3 as being the level required for receptive status. FCM findings were recorded in advance of ERA findings. Data collection and validation of the technique is ongoing but we wish to present our preliminary findings here.
Participants/materials, setting, methods: A standard stimulation regimen was employed across all participants with 5 days exogenous progesterone prior to pipelle biopsy on the sixth day (corresponding to approximately day 21–24 of the patient cycle). 4 patients were reassessed following 7 days progesterone and an original pre-receptive finding on ERA. Biopsies were split into ERA preservative solution and RPMI for FCM. The FCM tissue was mechanically dissociated and evaluated immediately using specific antibodies to epithelial cells and β3 integrin.
Main results and the role of chance: Using the ERA test, 14 patient samples were called pre-receptive, 12 were fully receptive and 1 was post-receptive. The almost 50:50 split between receptive and pre-receptive data sets is far higher than expected based on published ERA findings in a general population (~75% receptive vs. 25% non-receptive), indicating the origin of difficulties with IVF RM/IF patients may be multifactorial. Simultaneous assessment using the epithelial β3 integrin FCM technique showed good correlation between the tests. The greatest predictive value was in the 12 fully receptive patients where the two tests concurred 83% (10 out of 12) of the time. Of the 14 pre-receptive patients on ERA, 6 were actually interpreted as receptive using β3 expression alone. This latter decrease in the predictive value to approximately 57% concurrence may indicate the greater difficulty in assessing borderline cases using this technique. Subsequent cases will be assessed with a new benchmark of 65% epi b3 expression required to attain receptive status. 4 ERA assessed non-receptive cases were re-assessed following an additional 1–2 days of progesterone and were found to move into receptive status using both techniques indicating that the window of implantation can be relatively easily manipulated.
Limitations, reasons for caution: The study is ongoing and the data set is small. Set up and interpretation of FCM results is variable and may not yield the same results in another institution. The cut off value of 60% epithelial β3 expression was assigned based on initial ERA findings in 5 patients.
Wider implications of the findings: As well as its accepted role in assessing immune populations such as natural killer cells, T regulatory cells etc., in endometrial tissue the flow cytometer can give strong cost effective indications of receptivity status with application of the correct markers, acting as an entry level test employed prior to ERA.
Trial registration number: This is an observational study and not a clinical trial. Full patient consent obtained.

P-106 A one year retrospective analysis comparing live birth outcomes from embryos grown and transferred from an undisturbed time-lapse culture system with a conventional culture system

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Study question: Will an undisturbed time-lapse culture system influence an embryo's ability to grow, implant and yield a live birth?

Summary answer: An undisturbed environment yields embryos that are more advanced in development and an increase clinical pregnancy and live birth rate.

What is known already: With the introduction of novel time-lapse incubator technology (e.g., EmbryoScope™), embryos can be cultured in an undisturbed environment without being removed daily for observation. Meseguer (2011) initially showed an improved live birth rate using this system, however a review by Swain (2014) suggested that there is no one incubator type, undisturbed or otherwise, that yields better clinical pregnancy or live birth rates. Other studies (Kirkegaard, 2012; Park, 2015) have shown no improvement in embryo quality using undisturbed compared to conventional culture. Therefore the question of whether and how undisturbed culture is beneficial is not yet fully answered.

Study design, size, duration: A one year retrospective analysis of embryo growth (stage and grade) and live birth outcomes. The treatment group were the cycles cultured in undisturbed conditions (EmbryoScope™) (n = 317 cycles, 2468 embryos) compared to control cycles with removal for daily observation in a (Hunter G-185 Flatbed) incubator (n = 341 cycles, 2282 embryos). All cycles used Vitrolife sequential (G1+/G2+) media under 5% O_2. 98% of cycles used own gametes and embryos.

Participants/materials, setting, methods: Cycles with 3–12 zygotes on day 1 were allocated to EmbryoScope or Hunter incubators in a non-systematic manner depending on the number of cycles and availability of space on the day in each incubator. Embryos were transferred on day 3 or 5, with additional grading on day 2 (by removal from the Hunter incubators). Selection for transfer was by conventional grading, not using morpho kinetic time-lapse parameters in the EmbryoScope cycles.

Main results and the role of chance: Using Mann-Whitney and Fisher's statistical tests, it was found that cycles using the EmbryoScope/undisturbed culture had a significantly increased chance of a live birth (42% vs. 31%) (P = 0.006), as well as a significantly reduced chance of biochemical/early loss (4.4% vs. 11.1%) (P = 0.001) than those cycles cultured in the Hunter/Conventional culture system. Embryos also grew at a faster rate as there were more 4 cell embryos on day 2 and 8 cell embryos on day 3 in the EmbryoScope, compared to the Hunter group (P = 0.002). This suggests embryo development is more advanced in an undisturbed culture system. Differences in full embryo morphological grade (including cell evenness and fragmentation) requires further analysis.

The patient/cycle groups were similar between the two systems in terms of clinical parameters: treatment type (IVF/ICSI); Age; Day of transfer (3 or 5); and attempt number. The average number of zygotes was slightly higher in the treatment group (on average 1 embryo more, $p < 0.001$), however, when comparing cycles that yielded the same numbers of embryos, EmbryoScope resulted in improved live birth rates up to cycles that obtained 9 embryos. Above this, results are too low to compare.

Limitations, reasons for caution: The cycle allocation was not formally randomised. Patients who had fewer than 3 or more than 12 embryos were excluded from the study. Patients had slightly more fertilised embryos in treatment group. EmbryoScope cycles selected for blastocyst culture needed a G2+ media refresh on morning of day 3.

Wider implications of the findings: Our study suggests that undisturbed culture in EmbryoScope may improve treatment success, irrespective of the use of time-lapse selection algorithms. Conventional culture methods which cause disruption of the culture environment (atmospheric oxygen, pH, temperature, osmolality) to grade embryos during growth may negatively affect embryo growth and implantation potential.

Trial registration number: N/A.

P-107 Spontaneous abortion rates are decreased by using sperms following annexin V magnetic activated cell sorting (MACS) in ART for recurrent miscarriage patients

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Study question: Are sperms collected by annexin V MACS detrimental for spontaneous abortion in recurrent pregnancy loss couples during *in vitro* fertilization (IVF)?

Summary answer: when sperms collected by annexin-V-MACS are used for recurrent miscarriage couples during ART, spontaneous abortion rates are decreased and so, live birth rates are increased.

What is known already: About 12–15% of clinical pregnancies end in spontaneous abortion. Most pregnancy losses results from chromosomal or genetic, abnormalities, and random events. The abnormality may come from the egg and/or sperm.

Study design, size, duration: A total of 107 recurrent miscarriage patients were investigated in this study between September 2011 and December 2014. In this study, the recurrent miscarriage was defined as the occurrence of two or more consecutive pregnancy loss. The recurrent miscarriage couples were divided into two groups according to sperm preparation method. (Swim-up group: sperms were prepared with swim-up protocol; annexin-V-group: sperms were collected by annexin-V-MACS). Swim-up group included 37 cycles and annexin-V-group included 70 cycles.

Participants/materials, setting, methods: Once the sperm and anti-annexin-V antibody conjugated microbead are mixed for 15 min, the damaged sperm and microbead bind together. This complex cannot pass through the magnetized column, so we can prepare healthy sperms in the pass-through fraction. Finally the healthy sperms can be used for ICSI. We carried out tunnel-assay and halo-assay to examine DNA damage and apoptosis. We performed immunefluorescence staining using Annexin-V antibody on sperms to check the sorting-ability of Annexin-V column.

Main results and the role of chance: The results of halo test showed that the percentage of DNA damaged sperm were dramatically low in Annexin V group than swim-up group (4.3% versus 1.6%). In tunnel assay, we found that the number of apoptotic sperms is also lower in Annexin V group than swim-up group. In swim-up group, 6 cases out of 16 ongoing pregnancies were found to be miscarriage, and in Annexin V group, only 3 cases out of 29 ongoing pregnancies were found to be miscarriage (spontaneous abortion rates; swim-up versus annexin V group: 37.5 vs. 10.3 p = 0.031). In conclusion, Live birth rate in ART treatment for recurrent miscarriage patients were significantly higher in Annexin V group than swim-up group (Annexin group vs. swim-up group: 27.0 vs. 37.1).

Limitations, reasons for caution: A major limitation of this study is the small sample size, and our study was restricted to patients treated by GnRH antagonist protocol and so requires confirmation in a GnRH agonist protocol.

Wider implications of the findings: In recurrent pregnancy loss patients, using sperm collected by annexin V MACS can be an alternative solution to overcome miscarriage.

Trial registration number: None.

P-108 The correlations between chromosomal distribution of early pregnancy loss and the existence of an embryo as well as the postmortem embryonic pole length after IVF-ET

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Study question: To compare the chromosomal distribution in embryos from early pregnancy loss (EPL) with or without embryo after IVF-ET.

Summary answer: The chromosomal distribution differs in EPLs with or without embryo after IVF-ET.

What is known already: About 15% of clinical pregnancies will suffer EPL in natural conception and chromosomal abnormalities are the main causes. The miscarriage rate in IVF population is up to 24–30%. Three previous studies

have compared the chromosomal distribution in association with presence or absence of embryo, and the results were inconsistent. Furthermore, few researches have been conducted on the correlations between postmortem fetal pole length and chromosomal karyotypes.
Study design, size, duration: The data of 2172 women who underwent dilation and curettage (D&C) from January 2008 to December 2013 for missed abortion were retrospectively analyzed. The existence of an embryonic pole as well as the length of postmortem embryonic pole of embryos from EPLs were checked by transvaginal sonography (TVS). And ultrasound findings were compared with the karyotype results.
Participants/materials, setting, methods: This analysis included 2172 infertility patients who got singleton pregnancy and experienced EPL after IVF-ET. EPLs were divided into embryonic (1227) and anembryonic group (945) based on TVS diagnosis. The crown-rump length (CRL) of fetal pole (once observed) was measured twice for each fetus after confirmation of fetal death, subject to the final measurement before D&C. And the karyotype analysis was performed using comparative genomic hybridization (CGH) plus fluorescence in situ hybridization (FISH) technology.
Main results and the role of chance: In the 2172 cases, 1227 (56.49%) were found to be embryonic, and 945 (43.51%) were anembryonic miscarriages. The chromosomal abnormality rate was significantly higher in miscarriages with embryo than that of without embryo (52.24% vs. 35.35%, $p < 0.01$). Monosomies were found more frequent in embryonic than those in anembryonic group (6.76% vs. 3.81%, $p < 0.01$). The abnormal karyotype rate was significantly higher in the yolk sac only than that of in the empty sac group (43.39% vs. 28.11%, $p < 0.01$). There were statistically significant differences in the length of post-mortem embryonic pole among groups with different karyotypes. In addition, trisomy 21, monosomy X and triploidy were found with the longest length of postmortem embryonic pole. And longer than 20mm appears to be a prediction for trisomy 21 or monosomy X.
Limitations, reasons for caution: We used nonparametric test to compare the embryonic pole lengths due to non-normal distribution and non-homogeneous variances which may result from the limited cases of some rare chromosomal abnormalities. Another limitation was there was no mosaicismy contained in the cytogenetic results because CGH was unable to detect mosaicismy.
Wider implications of the findings: Chromosomal distribution in EPLs with embryo was different from that without embryo, and the postmortem fetal pole lengths were correlative with different karyotypes after IVF-ET. These findings may help clinicians to analyze possible causes when encountering these two kinds of miscarriages. CGH+FISH technology is reliable technology for screening the chromosomal anomaly quickly.
Trial registration number: None.

P-109 The effect of Fresh-ET and FET on maternal and neonatal outcomes

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Study question: To investigate whether the maternal high-E 2 environment and embryo cryopreservation increases the risks of maternal pregnancy complication and LBW and SGA birth.
Summary answer: The high estrogen level increases the incidence of SGA and LBW in fresh ET cycle and embryo freezing technology does not affect the birth weight.
What is known already: During a fresh IVF-ET cycle, exogenous gonadotropins are administered to promote the development of multiple follicles resulting in estradiol levels that can be 10–20 times greater than physiologic level. There have been increasing concerns that a disrupted endocrine environment may deteriorate the responses of gamete/embryo and impede embryonic development. It is shown that even singletons after ART are at increased risk for low birth weight (LBW), and singletons conceived through fresh ET have lower birth weight and more LBW than those through frozen ET.
Study design, size, duration: The clinical data were retrospective analyzed from patients treated with IVF/ICSI in the reproductive center of the third Affiliated Hospital of Zhengzhou University between January 2008 to September 2012. This study included 1336 patients who performed Fresh-ET (high-$E_{2\ group\ 587\ cases}$ and low-E_2 group 749 cases), 640 patients canceled transplant in fresh IVF cycle performed FET (FET group), all delivered singleton term infants. Naturally conceived singletons during the same period served as a control group ($n = 668$).
Participants/materials, setting, methods: The clinical data were retrospective analyzed from patients treated with IVF/ICSI in the reproductive center of the third Affiliated Hospital of Zhengzhou University between January 2008 to September 2012. This study included 1336 patients who performed Fresh-ET (high-$E_{2\ group\ 587\ cases}$ and low-E_2 group 749 cases),640 patients canceled transplant in fresh IVF cycle performed FET (FET group), all delivered singleton term infants. Naturally conceived singletons during the same period served as a control group ($n = 668$).
Main results and the role of chance: The live birth rate of FET group is higher than Fresh-ET group, difference was statistically significant ($P < 0.05$). The incidence of ectopic pregnancy rate, abortion rate, premature birth rate, pregnancy complications incidence in Fresh-ET group is higher than FET group, difference was statistically significant ($P < 0.05$). The pregnancy rate is similar between the two groups and has no statistically significance. The incidence of LBW and SAG was statistically significant between High-E_2 group and Low-E_2 group ($P < 0.05$), and the incidence of LBW and SGA in High-E_2 group was higher than that in Low-E2 group.
Limitations, reasons for caution: The clinical data were retrospective analyzed from patients treated with IVF/ICSI in the reproductive center of the third Affiliated Hospital of Zhengzhou University between January 2008 and September 2012, which is a cohort study, not randomized controlled.
Wider implications of the findings: Compared with the fresh ET cycle, FET increases the birth weight of the newborn, and pregnancy complications are reduced. The ectopic pregnancy rate, abortion rate, premature delivery rate, pregnancy complication rate of Fresh-ET group was higher than that of the FET group, and the live birth rate in FET group is higher than Fresh-ET group.
Trial registration number: 123456789.

P-110 Urinary human chorionic gonadotrophin (hCG) levels in early pregnancies correlate with serum hCG and may be used for the monitoring of early pregnancy well-being

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Study question: Do urinary hCG levels in early viable, ectopic and miscarried pregnancies correlate with serum hCG and represent a reliable marker of early pregnancy well-being?
Summary answer: Urinary hCG profiles in early pregnancy were representative of serum hCG for all studied outcomes and differed substantially between viable and ectopic/miscarried pregnancies.
What is known already: Quantitative assessment of serum (hCG) levels represents the gold standard for the monitoring of early pregnancy. However, this analysis is currently performed in diagnostic laboratories and cannot be carried out at the point of care. In addition, repeated blood tests can be inconvenient for patients requiring regular monitoring of serum hCG for suspected ectopic pregnancy, miscarriage or pregnancy of unknown location. Although qualitative urinary hCG tests represent a common and convenient tool for the diagnosis of pregnancy, it remains unclear whether quantitative assessment of urinary hCG could be used for the monitoring of early pregnancy well-being.
Study design, size, duration: An observational cohort study to monitor urinary and serum hCG levels was carried out in 82 female volunteers attending an early pregnancy unit (EPU). The monitoring was started on the day of referral and continued until 12 weeks pregnancy (since last menstrual period) for viable pregnancy or until resolution of an ectopic pregnancy/miscarriage diagnosis.
Participants/materials, setting, methods: The study recruited two groups of volunteers: women with a previous history of miscarriage or ectopic pregnancy (Group A, $n = 55$) and women with suspected ectopic pregnancy (Group B, $n = 27$). Urinary hCG levels were measured in the first morning urine samples using AutoDELFIA. Serum hCG levels were measured using an automated

immunoassay system. Urine creatinine was measured to examine effect of urine concentration correction.
Main results and the role of chance: Pregnancy outcome was classified as "viable" for 49 (59.8%) volunteers, "miscarriage" for 17 (20.7%), "ectopic pregnancy" for 11 (13.4%), "inconclusive" for four, and "missing" for one volunteer. Time profiles of log urinary hCG concentrations differed considerably for the three main pregnancy outcomes ("viable", "ectopic", "miscarriage"). Specifically, urinary hCG had a very similar time profile in volunteers with viable pregnancies, but differed substantially in those with ectopic pregnancies and miscarriages depending on the type and timing of the pregnancy outcome. For all three outcomes, urinary hCG concentrations rose steeply in the first days of pregnancy and reached a peak between 50 and 70 days before dropping off to varying degrees. Viable pregnancy profiles were best modelled by the critical exponential model. Miscarriage and ectopic pregnancy urinary hCG profiles were not modelled well, mainly due to inadequacies in the data, but appeared different to viable pregnancies. The study demonstrated a clear linear relationship between log urinary and log serum hCG levels that was common for all pregnancy outcomes and was judged to be valid. The slope between the two was estimated to be 1.006 with a correlation of 0.923.
Limitations, reasons for caution: The study did not include a sufficient number of volunteers with miscarriage and ectopic pregnancy, and there were indications of inadequate timing and frequency of data collection in these participants. Collectively, this was likely to affect performance of the models used to characterize urinary hCG profiles in these clinical scenarios.
Wider implications of the findings: The possibility to model urinary hCG profiles in viable pregnancies and consistency of the modelled profiles between participants opens new possibilities for the use of quantitative urinary hCG testing to differentiate between normal and compromised pregnancy development as an alternative to serum hCG testing.
Trial registration number: Not Applicable.

P-111 Miscarriage risk in women with twice, once and never poor response in two consecutive IVF treatment cycles.

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Study question: Does poor response in two consecutive IVF cycles increase the risk of miscarriage compared to once or never poor response?
Summary answer: Women with twice poor response more often experienced miscarriage compared to women with once and never poor response, independent of age.
What is known already: Poor response to ovarian hyperstimulation in IVF treatment is associated with an increased risk of miscarriage. Poor response may represent diminished ovarian reserve or may be accidental. Despite changes in hyperstimulation dosage or protocol, women with a diminished ovarian reserve easily experience a poor response in subsequent cycles as well. These women are hypothesized to have lower quality oocytes as compared to accidental poor responders. We hypothesize that women with twice poor response are therefore at an increased risk for miscarriage compared to women with once (accidental) or never poor response in two consecutive cycles.
Study design, size, duration: This study is part of the OMEGA-project, a Dutch nationwide retrospective cohort ($N = 19840$) including IVF-treated women between 1983 and 1995. All patients included completed two consecutive IVF-cycles within one year. Women treated with gamete or zygote intrafallopian transfer, ICSI, oocyte orembryo-donating were excluded, as were women with missing cycle information, extra-uterine or artificially terminated pregnancies.
Participants/materials, setting, methods: Patient's characteristics were obtained via questionnaires and medical records. Women achieving an ongoing pregnancy (viable intra-uterine pregnancy ≥16 weeks) after their second cycle were compared with those experiencing miscarriage (loss between 4 and 16 weeks). Poor response was defined as ≤3 oocytes and normal response as ≥4 oocytes. The frequency of poor response was divided into twice, once or never poor response. Odds ratios (OR) were calculated with logistic regression and categories were compared with trend analysis.
Main results and the role of chance: Complete data was available for 8457women, of which 5427 had 2 cycles, resulting in 1065 pregnancies. Of these, 773 ongoing pregnancies and 196 miscarriages we applicable for analysis. Women were on average 32.9 (±3.9) years and main causes of infertility included tubalfactor (37.3%), male factor (24.9%) and unexplained origin (22.3%). Twice poor response occurred in 4.4% (43/969) of the women, once in 17.4% (169/969) and never poor response in 78.1% (757/969). Women with twice poor response more often experienced miscarriage (37.2%) compared to women with once poor response (22.5%) (crude OR, 95% CI 2.0, 1.0–4.2; $P = 0.05$) and compared to never poor response (18.8%) (crude OR, 95%CI 2.6, 1.3–4.9; $P = 0.004$). Female age was identified as the only confounder to affect odds ratio materially (adjusted OR, 95%CI 2.3, 1.2–4.4; $P = 0.01$). There was no interaction between confounders including female age, response category, BMI and smoking. For the once poor responders, miscarriage rates were comparable between women experiencing poor response in their first or in their second IVF cycle (resp. 22.3% and 23.1%; $P = 0.92$). Our trend analysis showed a statistically significant decreasing risk among women with twice, once and never poor response ($P = 0.01$).
Limitations, reasons for caution: We analyzed an early dataset (1983–1995) and stimulation protocols were usually "short flare up" and "downregulation" protocols, therefore generalization should be cautious. Additionally, there was no information on ovarian reserve tests.
Wider implications of the findings: Women with twice poor response more often experienced miscarriage compared to women with once and never poor response, independent of age. This suggests that diminished ovarian reserve is related to lower oocyte quality. Our results are a valuable "prove of concept" of the relationship between poor response and oocyte quality.
Trial registration number: Not applicable.

P-112 Embryo transfer performed under ultrasound guidance can reduce the risk of caesarean scar pregnancy in women with isthmocele?

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Study question: Embryo transfer (ET) performed under 2D trans-abdominal sonography (2D-TAS) guidance can reduce the risk of caesarean scar pregnancy (CSP) during assisted reproductive treatments (ART)?
Summary answer: The location of the air bubbles assessed with 2D-TAS during ET didn't predict the site of implantation because other factors such as contractility were involved.
What is known already: The presence of an isthmocele, a triangular defect in the myometrium at the site of a previous caesarean delivery scar represent a risk factor for CSP and the incidence has risen with the increasing number of caesarian section deliveries performed. During ART, the possibility to identify with ultrasound the position of the catheter tip and/or the position of the air bubbles following embryo transfer could help the clinician to overcame the risk of CSP. However, it is possible that the embryo undergoes significant migration and the embryo position may change following transfer into the uterine cavity.
Study design, size, duration: A retrospective cohort study including 33 women with a previous caesarean section who underwent a 2D TAS guided ET at the Momò; Fertilife' Private Centre for Reproductive Medicine, Bisceglie, Italy, between August 2011 and October 2015.
Participants/materials, setting, methods: None of the patients had a ratio scar myometrial thickness/normal myometrial thickness ≤50%. At least one embryo of good quality (≥7 blastmomeres of equal size, with <10% fragmentation) was loaded into an atraumatic catheter and transferred into the middle of the uterine cavity under TAS guidance, aiming for an inner catheter tip placement of 15 mm from the fundus. In women achieving a pregnancy, the position of the gestational sac was subsequently assessed.

Main results and the role of chance: Over a period of four years, of 33 patients with secondary infertility and one previous cesarean section, 8 singleton and 2 double gestational sac were visualised after ART. Of these, 2 CSP were detected by transvaginal sonography (TVS) in the first trimester observing, respectively, one and two gestational sac with the "double ring sign" and the cardiac activity located anteriorly at the level of the internal os. The incidence of CSP was 1:540 (2/1080), which was much higher than spontaneous condition, suggesting ART could be a contributor to the occurrence of CSP. The twin pregnancy was treated with a dilatation and curettage under TVS guidance while the singleton pregnancy experienced a large amount of vaginal bleeding and a healthy boy weighing 2600 gr was delivered by an emergency caesarean section at 35 weeks' gestation.
Limitations, reasons for caution: Although the air bubbles seen at the time of ET are thought to demonstrate the position of the embryo, they are in fact a surrogate marker of the embryo itself, as this cannot be directly visualized by ultrasound scan.
Wider implications of the findings: The exact position of the embryo flash immediately following embryo transfer is not related to a good clinical outcome because the majority migrated towards the fundus or the cervix denoting an increased risk of embryo expulsion from the uterine cavity and its localization in an ectopic site such as isthmocele.
Trial registration number: None.

P-113 Embryonic aneuploidy: Is it responsible for the increased risk of early pregnancy loss in patients with PCOS?

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Study question: Is chromosome aneuploidy responsible for the increased risk of clinical miscarriage for women with polycystic ovary syndrome (PCOS)?
Summary answer: Abortuses of PCOS women are significantly less likely to have chromosome aneuploidy, independently of maternal age.
What is known already: It is widely known that the embryonic chromosomal anomalies are responsible for over 50% of early pregnancy loss after assisted reproductive technique. However, there is still a lack of knowledge about the cytogenetic information of embryos from abortuses of PCOS and no evidences were found to determine whether the increased risk of clinical miscarriage for PCOS women is due to embryonic aneuploidy or other endocrinologic factors.
Study design, size, duration: This prospective observational cohort study, between January 2010 and February 2014, followed up 1461 patients who conceived with *in vitro* fertilization and embryo transfer (IVF-ET). 100 patients diagnosed as clinical pregnancy loss were finally recruited including 32 with PCOS and 68 without PCOS.
Participants/materials, setting, methods: Cytogenetic analyses were performed by using cultured chromosome karyotyping and multiplex ligation-dependent probe amplification (MLPA) subtelomere assay combined with fluorescence in situ hybridization (FISH) before the year of 2013. Since 2013, diagnoses were made by using array-based comparative genomic hybridization (array-CGH) combined with cultured chromosome karyotyping. Incidence of chromosome aneuploidy in PCOS miscarriages was compared to non-PCOS miscarriages subjected to the Chi Square test. Predictive parameters for aneuploidy were tested in a logistic regression analysis.
Main results and the role of chance: There were 119 singleton clinical pregnancies in the PCOS group, of which 35 (29.4%) with follow-up miscarried; which compares to 11.3% (75/664) in the non-PCOS group, $P = 0.001$. No significant differences were found in the baseline characteristics between patients with and without PCOS, including maternal age, BMI, pregnancy history, gestational age, total dosages of gonadotropin for ovarian stimulation as well as methods of diagnosis. In PCOS group, 9 out of 32 (28.1%) abortuses demonstrated aneuploidy, which was significantly lower than Non-PCOS group (49 out of 68, 72.1%), $P < 0.01$. Maternal age was found to be an important predictor of aneuploidy; controlling for maternal age, abortuses of women with PCOS were significantly less likely to have chromosome aneuploidy (OR 0.14, 95% CI 0.05 to 0.39, $P = 0.001$).
Limitations, reasons for caution: Submicroscopic aberrations could be underestimated before the year of 2013 due to the unavailability of diagnoses made by using the array technique. To make firm conclusions on the incidence of embryonic chromosomal anomalies for PCOS miscarriages, a larger sample size diagnosed by using advanced sequencing technique is needed.
Wider implications of the findings: The present findings suggested hat the increased risk of clinical miscarriage for PCOS women is likely to be due to maternal factors other than aneuploidy, such as high testosterone level, anomalies in progesterone production and insulin resistant resulting in endometrial disorders.
Trial registration number: Empty.

P-114 Hydroxychloroquine treatment for recurrent pregnancy loss – a pilot study

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Study question: Can treatment with hydroxychloroquine (HCQ) 200 mg/day initiated prior to pregnancy increase the chance of a live birth in women with ≥4 unexplained pregnancy losses?
Summary answer: Among 15 HCQ treated women 12 (80%) have either ongoing second trimester pregnancies (8) or given birth (4). This is higher than expected 50%, $p = 0.035$
What is known already: Recurrent pregnancy loss (RPL) affects up to 3% of couples aiming for parenthood. RPL is associated with both physical and psychological short and long-term consequences. Half of the cases are unexplained with no proven treatments available. Fetal chromosomal abnormalities decrease with increasing number of losses and there is accumulating evidence that immunology plays a causal role in unexplained RPL. HCQ is a well-known anti-malaria drug with expanding indications in cancer and auto-immune diseases due to its anti-inflammatory, immune modulating and anti-thrombotic effects. HCQ is considered safe in pregnancy but its potential impact in unexplained RPL is unexplored.
Study design, size, duration: A pilot study in a prospective cohort of unexplained RPL with ≥4 losses. The cohort consists of 41 eligible women. Recruitment has taken place between October 1st 2014 and December 31th 2015.
Participants/materials, setting, methods: We included 41 eligible women from a Danish National Tertiary Referral Center for RPL. Tablet HCQ treatment of 200 mg/daily was administered orally after informed consent. Treatment was started preconceptionally and treatment was recommended 2 months before pregnancy and throughout pregnancy until gestational week 28. Women were informed about the HCQ treatment, contraindications, potential benefits and harm and follow-up procedures (Eyes cheked by ophthalmologist yearly, hemoglobin, platelets and leucocytes every third month during treatment).
Main results and the role of chance: Of the 41 women who initiated HCQ, four have given birth, eight are ongoing second trimester pregnant; four are first trimester pregnant, three lost their pregnancy and 22 are not pregnant yet. Among the 15 with ongoing second trimester pregnancy/live birth/pregnancy loss the median age is 37 (26–43), the median number of losses is 4 (4–10) and the median time of HCQ treatment prior to pregnancy is 2 months (0–5). The success rate for live birth/ongoing second trimester pregnancy is 12/15 (80%) which is significantly higher than the expected 50%, $p = 0.035$ (success rate in placebo group in a large randomized controlled trial of women with RPL and ≥4 losses). Treatment was initiated less than one month prior to pregnancy in all three pregnancies that failed. HCQ was well tolerated among the women. One complained about tinnitus but decided to continue treatment as the pregnancy sustained longer than ever before. Blood tests were unaffected by HCQ.

Although this is the largest cohort of HCQ treatment in unexplained RPL the low number of participants and the non-randomized design increases the risk that the results are due to chance. We expect the outcome numbers to increase at least 100% before ESHRE 2016.
Limitations, reasons for caution: The significant increase in live birth/ongoing second trimester pregnancies found in this prospective pilot study of HCQ treatment of unexplained RPL should be interpreted with caution due to the low number of participants and the non-randomized design.
Wider implications of the findings: There are no proven treatments to increase live birth rate in unexplained RPL. If the findings of this pilot study of HCQ

treatment in unexplained RPL patients can be confirmed in future randomized controlled trials, HCQ can be a well-tolerated, cheap and effective treatment of unexplained RPL.
Trial registration number: Pilot studies do not require registration according to Danish Legislation.

P-115 Maternal Killer-cell Immunoglobulin-like Receptor (KIR) and fetal HLA-C compatibility in ART-oocyte donor influences live birth rate

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Study question: Has the maternal KIR an impact in pregnancy, miscarriage and live birth rates (LBR)/cycle in donor oocytes –ART by paternal and oocyte donor HLA-C?
Summary answer: The combination of maternal KIR-parental, donors HLA-C, could predict which couple can benefit for donor selection by HLA-C, in order to increase the LBR.
What is known already: Pregnancies have an increased risk of recurrent miscarriage (RM), preeclampsia or fetal growth restriction (FGR) in mothers KIR AA when the fetus has more HLA-C2 genes than the mother. In human populations, pregnancy disorders are predicted to reduce the frequency of KIR A haplotype, HLA-C2, or both and this selection is thought to have originated during human evolution. In ART oocyte donor cycles, the oocyte HLA-C behaves as paternal HLA-C and the KIR-HLA-C combination is not taken into consideration nowadays during donors' selection. KIRAA women have lower live birth rate after doble embryo transfer (DET) in egg-donation ART cycles.
Study design, size, duration: Between January 2015 and December 2015, we performed a prospective study that included 30 women, with unknown etiology of their recurrent reproductive failure: recurrent implantation failure (RIF) ($N = 21$) and RM ($N = 9$) which had a total of 112 oocyte donor-assisted reproductive cycles (ART).
Participants/materials, setting, methods: Thirty patients undergoing to ART –oocyte donor, selected from our IVI Clinics, with normal karyotype (both members), trombophilic, and immunological results were studied prospectively. All women had KIR AA genotype and their partners HLA-C2 genes. They had 54 embryo transfer cycles with unknown HLA-C-oocyte donors and 28 cycles with HLA-C1C1 donors. Pregnancy, miscarriage and LBR/cycle after embryo transfer (ET) with unknown oocyte donor HLA-C and after transfers with HLA-C1C1 oocyte donor were studied.
Main results and the role of chance: The median age of our patients was 40 years and their body mass index ranged from 20.2 to 28.23 kg/m2. The median age of the patients' partners was 41 years. These couples had a mean of 3 previous failed egg donation ETs, and had been trying to conceive for a mean of 7+3 years. The median age of the oocyte donors was 25 years. From a total of 112 ART-oocyte donor cycles, 82 were included in the study; 30 cycles were not considered because the ETs were cancelled or not performed at the data collection time.

Higher pregnancy rate per cycle after HLA-C1C1 oocyte donor transfer (85.71%) compared with unknown HLA-C oocyte donor cycles (31.48%) were observed in the same patients KIR AA with HLA-C2 partners ($p < 0.0001$). Higher miscarriage rate per cycle after unknown HLA-C oocyte donor transfer (94.44%) compared with HLA-C1C1 oocyte donor transfer (8.33%) were observed ($p < 0.0001$).

Significantly increase LBR per cycle were observed after ET with HLA-C1C1 oocyte donor (82.14%) compared with the LBR in the same KIR AA patients-HLA-C2 partners after cycles with unknown HLA-C oocyte donor (0%) ($p < 0.0001$).
Limitations, reasons for caution: We must assume that is a small sample and that is the first report observing differences in LBR by oocyte donor HLA-C in mothers KIRAA with HLA C2 partners. However, apart from the statistical significance, the association strength is noticeably high, allowing greater confidence in the findings.
Wider implications of the findings: We speculate that completing a normal pregnancy is possible only for mothers KIR AA who carry a baby with a least one non–self HLA-C1. Therefore, selecting HLA-C1 amongst oocyte and/or sperm donors for patients undergoing to egg donation ART and KIR AA, could be more efficient and safer.
Trial registration number: Not applicable since no intervention has made.

P-116 Aneuploidy Screening and Genome Profiling in Couples with Spontaneous Recurrent Pregnancy Loss

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Study question: To investigate whether all genome sequencing by the involvement of specific genes can supplement sperm aneuploidy studies in patients with spontaneous recurrent pregnancy loss (RPL).
Summary answer: Gene sequencing confirmed a higher sperm aneuploidy and also evidenced mutations in a set of spermatogenesis-related genes in men that presented with RPL.
What is known already: At least 15% of infertility cases are related to genetic disorders, such as chromosomal and single-gene alterations. As a result, genetic tests are currently more frequently used as a diagnostic procedure in couples experiencing infertility or unexplained pregnancy loss. For this purpose, it is important to screen the male partner which is presently assessed by semen analysis, chromatin fragmentation, and fluorescent in situ hybridization (FISH) on spermatozoa. FISH is only capable of assessing a limited number of chromosomes, whereas all chromosomes assessment carried out by Next Generation Sequencing (NGS) may contribute to more proper profiling of the male genome.
Study design, size, duration: In a 14 month period, we assessed spermatozoa aneuploidy in 11 men with recurrent pregnancy losses. FISH analysis for chromosomes X, Y, 13, 15, 16, 17, 18, 21 and 22 was carried out. All chromosomes analysis was carried out by Next Generation Sequencing (NGS) and copy number variants (CNVs) were recorded to validate the chromosomal disomies involved. Genes with the highest CNVs were then noted for all chromosomes in each sample.
Participants/materials, setting, methods: FISH was performed on at least 1000 spermatozoa of 11 consenting men, with a threshold of >1.6% (euploid), maintaining a 2–3% error. Extracted DNA was amplified from at least 500 spermatozoa per sample using PCR-based random hexamer amplification yielding a DNA concentration of 447.8 ± 198ng/ul and quality of 1.7 ± 0.1nm. CNVs were recorded using CASAVA and VarScan2 software. The genes found to have the highest CNVs in each sample were selected and grouped by function.
Main results and the role of chance: A total of 11 men with an average age of 44.9 ± 7yrs had a semen specimen concentration of 27.0 ± 34 × 10^6/ml, motility of 23.0 ± 26%, and normal morphology of 1.5 ± 2%. The overall average aneuploidy by 9 chromosome FISH for those patients was 0.39%. When sperm aneuploidy assessment was carried out by CNV count, the aneuploidy rate rose to 4.07% ($P = 0.0001$). By NGS, we were able to detect a total of 14 putative gene mutations that were present in our study group. A few of these genes, ADAM3A, NXF2, RBMY1F, and DPY19L2, appear to be related to the development of a normal male gamete. When we assess the ability of these couples to reproduce with the help of assisted reproductive technology, only the couples treated with IVF and/or ICSI, but not IUI, were able to establish a pregnancy that resulted in a delivery of a healthy child.
Limitations, reasons for caution: Although this approach of assessing aneuploidy and gene mutations by NGS may certainly help in clarifying covert male genetic contributions involved in recurrent pregnancy loss, more cases need to be studied to further validate our findings.
Wider implications of the findings: The utilization of NGS for analysis of spermatozoa is beneficial to the assessment of aneuploidy, and detection of CNVs allow to screen for gene mutations. NGS may help identify specific genes to provide insight to underlying genetic causes of recurrent pregnancy loss, therefore clarifying the etiology of idiopathic male infertility.
Trial registration number: N/A.

P-117 Effects of previous ectopic pregnancies and the pertaining treatments on *in vitro* fertilization/intracytoplasmic sperm injection and frozen-thawed embryo transfer outcomes

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Study question: What are the influences of previous ectopic pregnancies and the pertaining treatments on *in vitro* fertilization/intracytoplasmic sperm injection (IVF/ICSI) and frozen-thawed embryo transfer outcomes?
Summary answer: Previous ectopic pregnancies (EPs) affect intrauterine implantation and EP rate.
What is known already: EP is an important cause of morbidity and mortality worldwide with unelucidated pathogenesis. In women who seek for the help of assisted reproductive technology, those with a history of EP can reach a proportion about 14%. A recent study reported that in fresh embryo transfer cycles of IVF/ICSI, previous EP and different treatments of it had no effect on the main IVF/ICSI outcomes, except an increased risk of recurrent EP. However, numerous studies suggested that frozen-thawed embryo transfer (FET) cycles were associated with a different endometrial environment and lower risk of EP compared with fresh embryo transfer cycles.
Study design, size, duration: This was a retrospective cohort study involving women undergoing 12,266 IVF/ICSI-FET cycles in the period between May 2013 and June 2015.
Participants/materials, setting, methods: Women with previous EP(s) and women got fallopian diseases without EP were strictly matched by age, number of cycles, type of infertility, presence of polycystic ovary syndrome, endometriosis and/or male factor infertility, and divided into four groups according to the times of previous EPs: one EP group (n = 959 cycles), recurrent EPs group (n = 375 cycles) and their control groups. Subgroups were made according to the stage of embryos transferred, endometrial preparation or treatment (surgical vs. non-surgical, rematched) of EP.
Main results and the role of chance: Main baseline characteristics and main ovarian stimulation outcomes had no statistically significant differences between experimental groups and their control groups, or between subgroups and their control groups, nor did the main pregnancy outcomes. However, the intrauterine implantation rate of recurrent EPs group (REG) (29.7% vs.36.6%, relative risk reduction [RRR]: 18.9%, 95% confidence interval [CI]: 5.5%–32.2%), cleavage stage embryo transfer subgroup (28.1% vs.34.7%, RRR: 19.0%, 95% CI: 4.6%–33.4%) and mild stimulation subgroup of REG (29.8% vs.45.1%, RRR: 33.9%, 95% CI: 13.7%–54.2%) were significant lower than their control groups. Meanwhile, the blastocyst forming rate in the blastocyst stage embryo transfer subgroup of REG was significant higher than its control group (27.0% vs.21.8%, relative risk increase [RRI]: 23.9%, 95% CI: 9.8%–37.9%). And a significant higher EP rate was observed in one EP group (6.4% vs.2.5%, RRI: 156.0%, 95% CI: 52.6%–259.4%), REG (5.3% vs.1.0%, RRI: 430.0%, 95% CI: 65.0%–795.0%) and the cleavage stage embryo transfer subgroup of REG (6.0% vs.1.2%, RRI: 400.0%, 95% CI: 54.2%–745.8%) than their control groups.
Limitations, reasons for caution: This was a retrospective study with thus inevitable selection bias. Moreover, the date was not derived from multiple centers. The sample size was quite limited, especially for the recurrent EPs group. And treatments of EP were performed in various hospitals. For surgical treatment, we didn't differentiate between salpingectomy and salpingostomy.
Wider implications of the findings: Women with a history of recurrent ectopic pregnancies have a compromised intrauterine implantation outcome after IVF/ICSI-FET, which to some extent indicates an altered endometrium that may play an important role in the pathogenesis of ectopic pregnancy. And for those women, blastocyst stage embryo transfer is suggested.
Trial registration number: Does not apply.

P-118 Primary recurrent miscarriage is linked to elevated peripheral natural killer cells

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Study question: Are altered levels of peripheral und uterine natural killer cells (pNK, uNK) present in patients with recurrent miscarriage (RM) and controls?
Summary answer: Compared to controls, higher pNK percentages were present in primary, but not in secondary RM and no correlation between pNK and uNK was detected.
What is known already: NK cells play an important role during early pregnancy and are part of immune diagnostics in RM. PNK and uNK can be distinguished according to their receptor expression and interaction with other players of the immune system. Recent studies indicate that elevated pNK and uNK might be associated with RM. However, controversial debates are ongoing as the comparison of the studies is difficult due to small cases included and inconsistent inclusion criteria.
Study design, size, duration: Between 10/2011 and 12/2015, in total n = 590 RM patients and 51 healthy female controls (regular menstrual cycle, no oral contraceptives) were included. Patients were screened according to a standard diagnostic protocol for anatomical, endocrine, autoimmune, hemostatic, genetic and immune disorders. Finally, n = 268 couples with ≥3 consecutive RM were identified. Subgroups consisted of n = 151 primary RM, n = 85 secondary RM, n = 147 idiopathic RM and n = 121 non-idiopathic RM patients.
Participants/materials, setting, methods: Diagnostic was performed in non-pregnant patients and controls in the mid-luteal phase. Peripheral blood levels of CD45+CD3−CD56+CD16+ NK cells were determined using four-color fluorescence flow cytometry and analyzed as a percentage of lymphocytes (%) and total NK concentration. In addition in n = 129 RM patients an uterine biopsy was taken during luteal phase to evaluate CD56+ NK cells by immunohistochemistry.
Main results and the role of chance: Patients with primary RM showed higher absolute pNK than patients with secondary RM (mean ± SD/µl 247.7 ± 123.8 vs. 205.7 ± 117.4, p = 0.01; 12.5% ± 5.7 vs. 11.1% ± 4.7, p = 0.04). In comparison to controls, there were higher NK percentages in idiopathic RM (controls: 10.5% vs. 12.1%, p = 0.02), non-idiopathic RM (12.1%, p = 0.04), primary RM (p = 0.007) but not in secondary RM patients (p = 0.40). With regard to uNK, there was no difference in uNK levels between the subgroups of RM and no correlation to pNK levels within the study population. However, seven idiopathic RM patients but none of the non-idiopathic RM patients showed strongly elevated uNK levels of >600/mm^2 (p = 0.06).
Limitations, reasons for caution: Whereas peripheral NK cells were studied in patients and controls, uterine biopsies for uNK cell analysis were only performed in patients but not in controls, limiting the conclusion.
Wider implications of the findings: A different immune etiology in primary compared to secondary RM might be responsible for altered pNK levels in these subgroups of RM patients. It seems that pNK and uNK are two distinct populations of immune cells and that both parameters need to be considered independently in RM patients.
Trial registration number: NA.

P-119 Multiple pregnancy after double embryo transfer is associated with a lower chance of miscarriage

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Study question: Are implantation rates (multiple pregnancy after double embryo transfer (DET)) associated with recurrent pregnancy loss (RPL) (two or more miscarriages)?
Summary answer: Women with RPL more often have lower implantation rates after DET.
What is known already: Angiogenesis is important in embryo implantation and recently it has been shown that RPL might be associated with increased instead of decreased angiogenesis. Women with RPL may be superfertile with as a result they may fail to discriminate between good and poor quality embryos, allowing implantation of all embryos including those with poor quality and consequently more often a clinical miscarriage as result. Therefore, we hypothesize that multiple pregnancy after DET resulting from optimal implantation might also associate with recurrent miscarriage both as implication of superfertility.
Study design, size, duration: We used the Dutch OMEGA cohort comprising 12,812 women who received at least one in-vitro fertilization (IVF) cycle

between 1983 and 2001. They were sent a questionnaire from 1997 till 2000 or from 2010 till 2013. Medical record data, including embryos transferred per woman per IVF cycle and the outcome, was linked to the questionnaire data. Number of miscarriages per woman was obtained from the women's questionnaires.

Participants/materials, setting, methods: Women were ordered according to their number of miscarriages in the following groups; zero miscarriages, one miscarriage and two or more miscarriages. Mean implantation rates were calculated from all IVF-cycles per woman. The association between mean implantation rate and total amount of miscarriages was estimated with an ordinal logistic regression. Sensitivity analyses were performed with implantation rate of each first IVF cycle per women and the maximum of all IVF cycles per woman.

Main results and the role of chance: There were 10,058 women (78.5%) without miscarriages, 1,922 women (15.0%) with one miscarriage and 832 women (6.5%) with two or more miscarriages. In the ordinal logistic regression we choose every step to be an increase of ten per cent. The odds ratio (OR) for mean implantation rate of all IVF cycles per woman was 0.86 (95% confidence interval (CI) 0.84 – 0.87). This means that for every ten per cent increase in implantation rate (for example from fifty per cent to sixty per cent) the OR of having RPL compared to having one miscarriage is 0.86 and this is the same for having one miscarriage compared to having zero miscarriages. ORs for first IVF cycle (0.92, 95% CI 0.90 – 0.93), for maximum implantation rate (0.91, 95% CI 0.90 – 0.93), for all women with a multiple embryo transfer (0.97, 95% CI 0.95 – 0.98) and for all women with a DET (0.96, 95% CI 0.94 – 0.97) were calculated. When excluding all nulliparous women ORs were similar, therefore all nulliparous women were kept in de final model.

Limitations, reasons for caution: Information about miscarriages was derived from the women's questionnaires and therefore may have been prone to recall bias. However, this is not very likely, because women probably were not aware of a possible association between implantation rate and miscarriages. Contribution of quality of embryos transferred could not be assessed.

Wider implications of the findings: Our results show that lower implantation rates are associated with RPL. Therefore either multiple pregnancy after DET or RPL is not representing superfertility. In contrast, based on the current findings a theoretical consequence may be that women with miscarriages may undergo DET with less risk of developing a multiple pregnancy.

Trial registration number: Not applicable.

P-120 Limitations of radiological screening tests in detection of subtle incomplete septum or arcuate uterine anomaly in patients with recurrent pregnancy loss (RPL)

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Study question: Evaluate the accuracy of hysterosalpingogram (HSG), trans-vaginal 2D ultrasound (2D US), and trans-vaginal 3D US in detecting subtle uterine anomaly in patients with RPL.

Summary answer: None of the radiological screening tests used in this study, alone or in combination, are accurate enough in detecting such anomalies in patients with RPL.

What is known already: In 50% of patients with RPL the etiology is unexplained. Our group has previously postulated that in infertile patients routine radiological tests are not accurate in the diagnosis of subtle uterine septum (Abuzeid et al.; ASRM, 2015; Corrado et al.; ASRM, 2013; Kallia et al.; ASRM, 2011). There is limited data on the association of subtle uterine septum and RPL and on the best method for detecting such anomalies. Hysteroscopic correction of significant uterine septum is associated with improved reproductive outcome in both infertile patients and patients with RPL.

Study design, size, duration: This is a retrospective study. The study included 120 consecutive patients with RPL who were found to have incomplete uterine septum (n = 62, 51.7%) or accurate anomaly (n = 58, 48.3%) on diagnostic hysteroscopy in the period between 1992 and 2014.

Participants/materials, setting, methods: The study included 120 RPL patients who were referred to our reproductive medicine unit in Flint, Michigan. We compared the findings on diagnostic hysteroscopy with the findings on various radiological tests that were used in the screening for such anomalies. Seventy five patients underwent HSG (62.5%); 109 patients underwent 2 D US (90.8%); 70 patients underwent 3 D US (58.3%); 79 patients underwent saline infusion hysterosonogram (SIH) with 3 D US (65.8%) before diagnostic hysteroscopy.

Main results and the role of chance: The diagnosis of subtle uterine septum or arcuate uterine anomaly was made on HSG in only 41 patients (54.7%). When 2 D US was used the diagnosis was made in 52 patients (47.7%). 3 D US was accurate in making the diagnosis in 42 patients (60.0%), while when SIH with 3 D US was used the diagnosis was correct in 49 patients (62.0%). When both HSG and 2D US were used (n = 68) the diagnosis was correct in 46 (67.6%). When both 3D US and 3D US with SIH were used (n = 62) the diagnosis was correct in 48 (77.4%).

Limitations, reasons for caution: One of the limitations of this study is its retrospective nature with the inherent bias of such design. Therefore further prospective studies with larger sample size are required to confirm our findings.

Wider implications of the findings: The data in this study suggest that some patients with unexplained RPL may in fact have subtle uterine anomaly. Therefore in patients with a history of RPL and negative radiologic tests a diagnostic hysteroscopy is the only reliable method to rule out subtle congenital anomalies of the uterine cavity.

Trial registration number: N/A.

P-121 The prevalence of mycoplasma and ureaplasma related chronic endometritis in women with a history of recurrent pregnancy loss (RPL)

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Study question: To determine the frequency of *Mycoplasma hominus* and *Ureaplasma urealyticum* associated chronic endometritis in 1,583 women with RPL compared to 263 infertile controls attending the same clinic.

Summary answer: *M. hominus* and *U. Urealyticum* cultures were positive significantly more in women with RPL 315/1583 (19.9%) than from infertile controls 27/263 (10.3%) [$p < 0.0001$].

What is known already: Evaluation of known causal factors in women with RPL reveals a possible etiology in 55% of patients (Jaslow, 2010). Untreated chronic endometritis may contribute to recurrent miscarriage ((McQueen, 2015). *Mycoplasma* and *ureaplasma* positive cultures are strongly associated with preterm delivery (Goldenberg, 2008). Diagnosis and treatment of *mycoplasma* following a first or later pregnancy loss significantly reduces pregnancy loss in the next pregnancy (Quinn, 1983). *Mycoplasma* and *ureaplasma* infections have been a proven cause of bovine abortion for over 35 years (Doig, 1981).

Study design, size, duration: Single-center, retrospective cohort study of 1,846 women from 2005 to 2015. RPL cases (1,583) and infertile controls (263) were compared. RPL evaluation included karyotypes on both partners, uterine cavity evaluation, lupus anticoagulant, antiphospholipid antibodies, TSH, HgbA1c, progesterone, and Factor V Leiden. Infertile couples had a hysterosalpingogram, semen analysis, and documentation of ovulation. All women had endocervical cultures for *M. hominus* and *U. urealyticum* on selective media. Positive cultures were considered abnormal.

Participants/materials, setting, methods: All women attended the same reproductive center. They were matched 6:1 (cases = RPL to controls = infertile) based on age (mean 32.6 years; range 20 to 42 years), race (72% white, 26% African, 2% Asian and other), BMI (mean 27.1), and socio-economic status. All cultures were obtained in selective transport media and sent to the same laboratory. Positive cultures required treatment of both partners with doxycycline 100mg BID for 14 days with test of cure cultures after four weeks.

Main results and the role of chance:

Table.

	Infertile controls	Recurrent loss	*p* Value	Relative risk (95% CI)
Number	263	1583		
Positive *M. hominus*	1 (0.01%)	66 (4.2%)	0.0172	10.9 (1.53–78.7)
Positive *U urealyticum*	26 (9.89%)	249 (15.7%)	0.0147	1.59 (1.09–2.33)
Positive one or both	27 (10.3%)	315 (20.0%)	0.0005	1.94 (1.34–2.81)
Negative	236 (89.7%)	1268 (80.0%)	0.0005	1.94 (1.34–2.81)

Significantly more women with RPL (315/1583); 20.0%) were colonized with *mycoplasma* and/or *ureaplasma* than infertile controls (27/236; 10.3%) [$p = 0.0005$]. When both partners were treated with doxycycline for two weeks the test of cure four weeks later was negative over 90% of the time when treating *mycoplasma* and over 80% of the time when treating *ureaplasma*. Resistant patients were treated with levofloxacin or erythromycin to achieve a final cure rate of 95%. All others had a daily suppressive dose of erythromycin during pregnancy. The strengths of this study are that patients and controls are matched, attended the same clinic, had cultures obtained from the same laboratory, had standard evaluations for RPL and infertility. The study was powered with 95% confidence to detect a 5% difference in positive culture results.

Limitations, reasons for caution: Based on the power analysis, the role of chance is very small but present. The limitations of the study include: it was not prospective in design, all couples with a positive culture were offered treatment, however 40% of women did not complete treatment or were lost to follow up.

Wider implications of the findings: Women who are colonized with mycoplasma and/or ureaplasma generally do not have symptoms of vaginal irritation, discharge, rash, or odor. Cultures to identify these pathogens are readily available, easily and economically treated, and may provide an answer to many couples with previously unexplained RPL.

Trial registration number: Not applicable.

References:

Jaslow (2010). *Fertil Steril* 93:1234.
McQueen (2015). *Fertil Steril* 104:927.
Goldenberg (2008). *AJOG* 198:43.
Quinn (1983). *AJOG* 145:239.
Doig (1981). *Can Vet J* 22:339.

P-122 Immunomodulation of the endometrium using oral prednisolone in unexplained recurrent miscarriage and normal or high level of uterine natural killer cell density in the endometrium

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Study question: Are uterine Natural Killer (uNK) cells involved in the aetiology of unexplained recurrent miscarriage? And if so, is immunomodulation of the endometrium using prednisolone effective with unexplained recurrent miscarriage?

Summary answer: High uNk cell density is associated with recurrent miscarriage, prednisolone can be used as an immunomodulatory treatment in unexplained recurrent miscarriage to improve pregnancy outcome.

What is known already: Recurrent miscarriage is the loss of three or more consecutive pregnancies**.** Despite a wide range of investigations, no apparent cause is found in more than 50% of women with recurrent miscarriage and they are categorized as idiopathic or unexplained recurrent miscarriage**.** Uterine Natural Killer (uNK) cells are the most predominant leucocytes in the endometrium and their density varies throughout the menstrual cycle. UNK cell density increases in number towards the mid-luteal phase and peaks in early pregnancy if implantation occurs. Increased density of uNK cells in pre-implantation endometrium has been found in women with recurrent miscarriage compared to fertile controls

Study design, size, duration: A randomized controlled trial was conducted on two hundreds and four women with unexplained recurrent miscarriage during the period between January 2014 and December 2015.

Participants/materials, setting, methods: 204women were recruited from the Recurrent Miscarriage Clinic. Endometrial samples were taken for immunohistochemical assay uNK cells. Then divided into two groups according to the uNK cells density in the endometrium: -Group(I): women with uNK cells ≥5%. -Group(II): uNK cells < 5%.

Then follow up to 12 months, till they spontaneously got pregnant. Then they were randomized-Subgroup(A) received oral prednisolone in addition to the standard empiric treatment. -Subgroups(B) received the standard empiric treatment only.

Main results and the role of chance: 154 women had an uNK percentage ≥5%, while 50 had an uNK percentage < 5%. This means that increased density of uNK in pre-implantation endometrium is associated with recurrent miscarriage and may predict subsequent miscarriage. The rates of first-trimester miscarriage were comparable in women of subgroups Ia and Ib. This means similar risk of first-trimester miscarriage. The rates of live-birth were lower in subgroup Ia when compared to subgroup Ib; the difference was however, statistically insignificant. The rates of second trimester miscarriage were significantly higher in women of group II when compared to women of group I (threefold higher rate of second trimester miscarriage). Pregnancy-induced hypertension has similar rates between four subgroups. Also, the results showed that higher uNK cell density in preimplantation endometrium is associated with FGR and immunomodulation of the endometrium using oral steroids is associated with lower rate of FGR, although this association is not statistically significant. Also, the prevalence of gestational DM in our study is 6.5% ($n = 153$, 10 (6.5%)) which is consistent with its prevalence in a given population or ethnic group.

Limitations, reasons for caution: To our best knowledge, no more studies in the literature were found comparing oral prednisolone use in women with unexplained recurrent miscarriage with high or normal level of uNK cell density in the endometrium and the prognosis of pregnancy outcome which made our study results difficult to interprete.

Wider implications of the findings: We have demonstrated that high uNK cell density in preimplantation endometrium is associated with recurrent miscarriage and may predict subsequent miscarriage and administration of oral prednisolone in comparison to standard empiric treatment is not associated with significantly improving pregnancy outcome.

Trial registration number: We are in the process of registration on www.clinicaltrials.gov.

P-123 Recurrent pregnancy loss – what is the impact of consecutive versus non-consecutive losses?

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Study question: Is the prognostic impact of consecutive early pregnancy losses different from that of non-consecutive losses in women with secondary recurrent pregnancy losses (RPL)?

Summary answer: Only consecutive early pregnancy losses after the last live birth exhibit a statistically significant negative prognostic impact in women with secondary RPL.

What is known already: There is consensus that the risk for new pregnancy loss increases with the number of previous pregnancy losses in patients with RPL and that second trimester losses exhibit a stronger negative impact than early losses. It is unknown whether the sequence of early pregnancy losses plays a role for the prognosis in patients with a prior birth.

Study design, size, duration: Retrospective cohort study of pregnancy outcome in patients with unexplained secondary RPL included in three previously published Danish double-blinded placebo-controlled trials of intravenous immunoglobulin (IvIg) conducted from 1991 to 2014. No other treatment was given. In two of the trials only patients with a minimum of four previous pregnancy losses were included. Six patients with documented explained pregnancy losses (clinical ectopic pregnancies and aneuploid miscarriages) in the trials were excluded.

Participants/materials, setting, methods: Among a total of 168 patients included, 127 had secondary RPL of whom 52.8% had a subsequent pregnancy loss. Data were analyzed by multivariate analysis including the independent variables: age, number of early pregnancy losses before and after the last live birth, respectively; a second trimester pregnancy loss before or after the last live birth, respectively; number of live births and presence of stillbirth. The outcome variable was unexplained loss in the first subsequent pregnancy.

Main results and the role of chance: In women with secondary RPL both a late and each early loss happening before the last live birth did not significantly impact the risk of new pregnancy loss in the trials: RR 1.31 (95% CI 0.62–2.77, $p = 0.48$) and RR 0.88 (95% CI 0.70–1.11, $p = 0.29$), respectively. In

contrast, the impact on risk of pregnancy loss during participation in the trials conferred by a late and each early pregnancy loss happening after the live birth was significant: RR 2.15 (95% CI 1.57–2.94, $p < 0.0001$) and RR 1.14 (95% CI 1.04–1.24, $p = 0.002$), respectively. None of the other independent variables exhibited significant impact on pregnancy outcome.
Limitations, reasons for caution: Forty-eight percent of the patients were treated with IvIg, which could in principle influence the results. However, allocation to IvIg was completely random and prognostic variables were equally distributed in IvIg and placebo treated patients. The number of patients was limited.
Wider implications of the findings: The finding that a live birth eradicates the negative prognostic impact of pregnancy losses happening before the birth in patients with secondary RPL has importance for our understanding of the pathogenesis. It emphasizes that only consecutive pregnancy losses not intervened by a birth should count in the definition of RPL.
Trial registration number: In the third Ivig trial: NCT00722475.

P-124 Effect of JAR spheroids on endometrial stromal cell decidualization

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Study question: To study the cell cycle in endometrial stromal cell decidualization and the effect of JAR spheroids on endometrial cell decidualization.
Summary answer: Decidualized endometrial stromal cells break through the cell cycle arrest in S phase after co-cultured with JAR spheroids and prepare for embryo implantation.
What is known already: Decidualization is a critical step during embryo implantation and characterized by the differentiation of endometrial stromal cells (ESCs) into decidual cells. Estrogen and progestogen promote endometrial stromal cell exit from the cell cycle and enter differentiation. More cells arrest in G0/G1 phase. Cell cycle inhibitor P27, P57 expression are improved in decimalized endometrial stromal cells.
Study design, size, duration: The ESCs from normal proliferative endometrial tissues (five patients) were isolated from normal cycling women by endometrial biopsy at the time of diagnostic laparoscopy for fallopian tube obstruction. Histological examination revealed normal endometrium. All cells were used at the third passage.

After induced decidualization with 0.5 mM 8-Br-cAMP and 10^{-6} M MPA for four days, ESCs are co-cultured with JAR spheroids for 2h in a incubator with a shaking table to avoid the spheroids attachment.
Participants/materials, setting, methods: The ESCs from normal proliferative endometrial tissues (five patients) were isolated from normal cycling women by endometrial biopsy at the time of diagnostic laparoscopy for fallopian tube obstruction. Histological examination revealed normal endometrium.
Reproductive center: RT-PCR western blot, immunofluorescence.
Main results and the role of chance: After co-cultured with JAR spheroids, P27, P57 expression in endometrial stromal cell are decreased when compared with the decidualization group ($P < 0.05$) both in mRNA and protein level. The expression of LIF and integrin beta3 are improved when compared with the decidualization group. JAR spheroids induce decidualized ESCs break through the arrest of cell cycle and prepare for embryo implantation/JAR spheroids attachment.
Limitations, reasons for caution: JAR spheroids could not replace real embryos.
Wider implications of the findings: The mechanism of the embryo implantation is subtle, and decidualization of ESCs is critical. We explained the cell cycle change of ESCs before JAR spheroids attachment and might help with the exploration of the dialogue between embryos and ESCs.
Trial registration number: None.

P-125 Expression of TRPV4 exposed to estrogen in the pathophysiology of tubal ectopic pregnancy

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Study question: The role of estrogen in the motility of fallopian tube cilia may lead to tubal ectopic pregnancy.
Summary answer: High level of estrogen acting through the motility of cilia on fallopian tube, may potentially lead to tubal ectopic pregnancy.
What is known already: The role of estrogen in the pathophysiology of tubal ectopic pregnancy.
Study design, size, duration: Open-label, non-randomized trial. The fallopian tubes were collected from five women with tubal pregnancy and five multiparity women with tubal sterilization from 2013 to 2014.
Participants/materials, setting, methods: The fallopian tubes were collected from five women with tubal pregnancy and five multi-parity women with tubal sterilization. The TRPV family were measured by quantitative real time polymerase chain reaction. The mRNA and protein expressions of estrogen and progesterone receptor in the ectopic implantation site and chorionic villi were examined. Moreover, we investigated the biological effects of estrogen and progesterone on TRPV4 using epithelial ovarian cancer SKOV3 cells.
Main results and the role of chance: TRPV4 expression was significantly decreased in ectopic implantation site and chorionic villi compared with normal fallopian tube tissues. Lower expression of TRPV4 in SKOV3 cells was associated with the concentration of estrogen ($P < 0.05$). In contrast, TRPV4 expression was significantly increased in high level of progesterone.
Limitations, reasons for caution: Limitation: We substituted the epithelial ovarian cancer SKOV3 cells for the normal fallopian tube cells. The physiology of cells might be different.
Wider implications of the findings: Estrogen and progesterone signals might contribute to the pathology of tubal cilia function by the expression of TRPV4 and provide better understanding on their respective roles in fallopian tube physiology and tubal ectopic pregnancy pathophysiology and etiology.
Trial registration number: Not clinical trial.

P-126 Identifying maternal constraints on fetal growth and subsequent perinatal outcomes using a multiple embryo implantation model

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Study question: We question whether adverse perinatal outcomes associated with assisted reproductive techniques (ART) occur as a result of epigenetic insults or due to maternal physiologic adaptations.
Summary answer: Our findings suggest that embryonic implantation sites during early gestation set the growth profile of each embryo, dictating later growth patterns and the gestational length.
What is known already: ART contributes to 1–2% of all live U.S. births annually. In recent years, there has been concern about the safety of ART, particularly *in vitro* fertilization (IVF). While the majority of singleton births after IVF are uncomplicated, studies have suggested that IVF pregnancies may be independently associated with increased risks of low birth weight (LBW), preterm birth, and perinatal mortality. These outcomes complicate multiple gestations as expected, but have also been reported in singleton conceptions. A multiple embryo implantation model allows for assessment of the early in utero environment, and therefore, assessment of any maternal constraints on developing fetuses.
Study design, size, duration: Retrospective, single-center study of ART cycles during a 16-year period were categorized according to the number of embryos transferred, those that implanted, and the actual number of live births. For each positive pregnancy test 10–15 days after embryo transfer, an ultrasonogram was performed to record the number of gestational sacs, fetal poles, and cardiac activity for each individual implanted embryo at 6–7 gestational weeks.
Participants/materials, setting, methods: Data from patients who were treated with ART, specifically intracytoplasmic sperm injection (ICSI), were collected. Controlled ovarian stimulation (COS), hCG trigger, oocyte retrieval and sperm injection were performed as per our standard protocols. First trimester implantation sites that resulted in live births were defined as "true" to distinguish them from those that spontaneously reduced called "virtual." All pregnancies with selective reduction were excluded. Birth outcomes analyzed included birth weight and gestational age at delivery.
Main results and the role of chance: A total of 17,415 cycles were analyzed. The average maternal age was 36.9 (±5.0) years. An overall fertilization rate of

73.4% generated approximately 48,708 good quality cleavage-stage embryos. In most patients (92.8%), an average of 3 embryos was transferred. The clinical pregnancy rate was 39.2% (n = 6,281). The overall occurrence of multiple gestations was 38.2% (n = 2,608) consisting of 2,038 twin, 511 triplet, and 59 quadruplet pregnancies. Of these multiple gestations, 18.6% of twin, 54.2% of triplet and 76.3% of quadruplet gestations spontaneously reduced. Failure of the implanted embryo to progress was not related to maternal age, embryo morphology or quality. Singleton newborns resulting from multiple implantation sites had lower birth weights (P < 0.01) and shorter gestational ages (P < 0.01) than those from a single implanted embryo. The number of embryos transferred did not affect the gestational length of singleton newborns. However, when more than one embryo implantation site was established, the proportion of births prior to 37 weeks of gestational age increased progressively (P < 0.01). Although the birth weights of singletons from multiple implantation sites (virtual singletons) were lower than true singletons, the birth weight of virtual singletons were comparable to the birth weights of true twin, triplet, and quadruplet live births.

Limitations, reasons for caution: While our study suggests that multiple embryo implantation is associated with lower birth weights and shorter gestational ages in singleton newborns, it must be noted that a myriad of maternal and uteroplacental factors ultimately determine in utero growth and development of fetuses.

Wider implications of the findings: Our study highlights that early in utero stress represented by multiple embryo implantations confers greater perinatal risk to the surviving fetus. Given the correlation between adverse perinatal outcomes and adult cardiovascular disease, diabetes, and dyslipidemia, avoidance of such stressors by reducing the number of embryos transferred assumes paramount importance.

Trial registration number: Not applicable.

P-127 The role of adjuvant treatment in unexplained recurrent miscarriage

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Study question: To assess the role of adjuvant treatment on improving outcomes after recurrent miscarriage (RM).

Summary answer: Empirical adjuvant treatment for the management of unexplained RM does not appear to offer any benefit. Prognosis with early pregnancy support alone is good.

What is known already: RM affects 1% of couples and is associated with significant psychological distress. Based on the current evidence base, RM patients are investigated for anti-phospholipid syndrome, karyotyping, structural abnormalities of the uterus and for patients with second trimester losses, thrombophilia. While there is no definitive treatment that can be offered in unexplained RM, various treatments including progesterone, aspirin, low molecular weight heparin and corticosteroids are often prescribed by clinicians, despite a lack of supporting evidence. This is because of the fact that patients who suffer RM are a vulnerable population, often desperate to try any adjuvant treatments suggested.

Study design, size, duration: A review of all patients (N = 301) attending recurrent miscarriage clinic between May 2013 and January 2015, to allow a minimum of one year follow up, was undertaken at two large teaching hospitals in the UK. Inclusion criteria specified that only women who had suffered ≥3 consecutive miscarriages were included. Patients with <3 consecutive miscarriages or with incomplete follow up data were excluded leaving a cohort of 242 patients.

Participants/materials, setting, methods: Data including demographics, past medical history, results of screening tests, adjuvant treatments given and outcomes of subsequent pregnancies were collected from the hospital patient records and entered onto a spreadsheet database. A statistical Package for the Social Sciences was used to analyse the relationship between demographic data and the outcome data. Multiple logistical regression analysis was used to assess the effect of variables on future pregnancy outcomes. P-value of < 0.05 was statistically significant.

Main results and the role of chance: 33.1% of women had abnormal investigations and 66.9% had an unexplained recurrent miscarriage. While the chances of having a live birth in both the unexplained (74.2%) and explained (64.5%) groups were similar (P = 0.35), empirical adjuvant treatment for unexplained RM did not confer any benefit compared to expectant management alone (67.9% vs. 76.7% respectively; P = 0.5). The prevalence of antiphospholipid syndrome (lupus anticoagulant, anticardiolipin antibodies and anti-Beta2 glycoprotein-I antibodies), thrombophilia, thyroid disease, parental karyotype abnormalities and structural uterine abnormalities were 7.4%, 4.5%, 6.6%, 2.9% and 6.6% respectively. Among all the variables (age, number of previous miscarriages, smoking or ethnicity) analysed, age alone was the significant predictor of future live birth (OR: 0.94; 95% CI: 0.94; 0.888–0.997; P < 0.05) and of the possibility of pregnancy within one year of follow up (OR: 0.936; 95% CI: 0.936; 0.89–0.998; P < 0.05).

Limitations, reasons for caution: While the study design was an observational cohort study, it had a diverse population of patients from two large teaching hospitals. We were unable to adjust for confounding factors, with some patients being screened positive for more than one abnormal test and some taking more than one empirical adjuvant treatment.

Wider implications of the findings: More than three quarters of women with a history of unexplained RM will have a live birth in their subsequent pregnancy with conservative management alone. Patients should be informed of this and the practice of prescribing adjuvant treatment should be restricted except in the context of a large RCT.

Trial registration number: N/A.

P-128 Antimullerian hormone as predictor of miscarriage in unexplained repeat pregnancy loss women under 35 years old

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Study question: The aim of this study was to evaluate if antimullerian hormone (AMH) was associated with pregnancy loss in young unexplained repeat pregnancy loss (RPL) women.

Summary answer: AMH values were not associated with repeat pregnancy loss in unexplained RPL women under 35 years old.

What is known already: Genetic cause, anatomic cause, and antiphospholipid syndrome are the only undisputed causes of RPL. Approximately 50% of cases of RPL are classified as unexplained. Advanced maternal age is related with miscarriages, which is predisposed to a greater risk of fetal aneuploidy. It has been proposed biological ovarian age is more important than chronologic age. AMH is well correlated with diminished ovarian reserve, readily used for predicting the oocytes' quantity in IVF cycle. Some studies regarding the relationship between AMH and miscarriage have been tried. However, the prospective study and evidence is still lack, especially in young women.

Study design, size, duration: Ninety nine women suffered three more miscarriage were consecutively registered as unexplained RPL after excluding the genetic, anatomic, endocrinological cause from January 2014 through December 2015. After excluding women over 35 years, 69 women diagnosed as unexplained RPL were enrolled.

Participants/materials, setting, methods: Maternal serum AMH levels were defined as low (< 1.0 ng/ml), normal (1.0–3.5 ng/ml) and high (> 3.5 ng/ml). To compare other etiological causes, Natural killer cell proportion, protein C activity, protein S activity, antithrombin III, homocystein and plasminogen activator inhibitor-1 (PAI-1) were evaluated. Pregnancy rate and miscarriage rate were compared among the groups. All spontaneous abortus were performed the cytogenetic study for checking the aneuploidy.

Main results and the role of chance: Mean age of each groups were not different as 33.75 ± 1.26 in low AMH, 31.95 ± 1.83 in normal AMH, and 31.02 ± 2.95 in high AMH group. Parity and previous miscarriage number were not different among the groups either. Natural killer cell proportion was elevated in 34 women. 8 cases of protein S deficiency and 3 cases of hyperhomocysteinemia were found. No significant difference was among the groups. And also, mean value of NK cell proportion, protein C activity, protein S activity, antithrombin

III, homocystein and PAI-1 were similar. Pregnancy rate in normal AMH group was 9/21 (42.9%), similar with 19/44 (43.2%) of high AMH group. Miscarriage rates were also similar between normal and high AMH group such as 33.3%, and 36.8% respectively. In low AMH group, only one women could be pregnant and suffered miscarriage again. Among total 11 abortus, 4 fetus were revealed as aneuploidy.

Limitations, reasons for caution: Because of the limited number of this study, further longitudinal studies with larger sample sizes are required.

Wider implications of the findings: The relationship of AMH and miscarriage is still unclear. It is important to find that the preconceptinal biological marker to predict the risk of miscarriage in a future pregnancy. It could be used for family planning and counselling.

Trial registration number: NO 12-262

P-129 Serum and tissue concentration of vascular endothelial growth factor (VEGF) in ampullary ectopic pregnancy and the depth of trophoblastic invasion

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Study question: To evaluate the correlation between serum concentration and tissue expression of VEGF and to associate the depth of trophoblastic invasion into the tubal wall with tissue VEGF in ampullary pregnancies

Summary answer: The depth of trophoblastic penetration into the tubal wall is not associated with tissue VEGF expression. Serum VEGF do not show correlation with tissue expression

What is known already: The implantation site in the tubal wall is different from well-vascularized endometrium and the concentration of VEGF seems to be elevated in ectopic pregnancy to accommodate to this unfavorable environment. Previous studies showed that higher serum VEGF concentrations is related to deeper invasion of trophoblastic tissue into the tubal wall.

Since there is greater expression of VEGF in ectopic pregnancy implantation site and that higher serum levels of VEGF are found in pregnancies with deeper trophoblastic invasion and, VEGF tissue concentration would also be correlated with the depth of trophoblastic invasion into the wall of the oviduct.

Study design, size, duration: A prospective study was conducted on patients with a diagnosis of tubal pregnancy in the ampullary region who were submitted to salpingectomy between July 11th, 2012 and August 19th, 2013.

Inclusion criteria were spontaneous conceived singleton pregnancies, diagnosis of tubal pregnancy in ampullary portion, radical surgical treatment (salpingectomy), and measurement of serum VEGF.

Exclusion criteria were: not ampullary pregnancy, impossibility of collecting blood sample, impossibility of either anatomopathological or tissue VEGF analysis.

Participants/materials, setting, methods: 63 cases were recorded. Forty two patients fulfilled the inclusion criteria and then 8 were excluded. Blood samples were collected to determine serum VEGF concentration with Luminex. Histologically, trophoblastic invasion into the tubal wall was classified as grade I when limited to the tubal mucosa, grade II when reaching the muscle layer and grade III when comprising the full thickness of the tubal wall. Tissue VEGF expression was measured by immunohistocemistry and point counting technique.

Main results and the role of chance: Histological analysis showed that 8 patients (23.5%) had stage I tubal infiltration, 7 (20.6%) had stage II, and 19 (55.9%) had stage III. The gestational age ranged from 28 to 95 days (53.2 ± 14.5 days), and there was no significant difference in mean gestational ages among the three histological groups ($p = 0.604$).

Serum VEGF concentrations on the day of surgery ranged from 0.36 to 205.16 pg/mL (median 14.77 pg/mL) and the median tissue VEGF percentage was 16.07 (range, 3.44–50.14). According to Kruskal-Wallis' test, the difference between the percentage of tissue VEGF was not significant in relation to the degree of trophoblastic invasion ($p = 0.621$).

Serum levels of VEGF did not show any correlation to its tissue expression. The Spearman's coefficient (Rho = –0.057) presented a weak correlation between these two variables ($p = 0.748$).

Multivariate logistic regression analysis was performed and the variable tissue VEGF was included in order to compare its performance as a predictive factor of depth of trophoblastic invasion into oviduct wall. Grade I versus II and III ($p = 0.321$) were compared and III versus I and II ($p = 0.230$). We observed that tissue VEGF showed no statistical difference for prediction of both degrees of trophoblastic invasion

Limitations, reasons for caution: no limitation or reasons for caution.

Wider implications of the findings: The percentage of tissue VEGF was similar in the three degrees of trophoblastic invasion. Different from previous studies, this finding suggests that VEGF at the site of tubal implantation appears not to be related to trophoblastic invasion and maybe other still unknown growth factors may contribute to this process.

Trial registration number: no trial registration number.

P-130 Abnormal human chorionic gonadotropin (hCG) trends in viable singleton pregnancies after transfer of multiple embryos

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Study question: Among viable singleton pregnancies, is the incidence of an abnormal early hCG rise increased following transfer of multiple embryos compared to single embryo transfer (ET)?

Summary answer: Abnormal hCG rises were significantly more common in singleton pregnancies after transfer of two or more embryos.

What is known already: Serial serum hCG levels are used to monitor early pregnancies following *in vitro* fertilization (IVF) treatment. An abnormal hCG trend, commonly defined as a rise less than 66% in 2 days, is concerning for a failing or ectopic pregnancy. This finding generates significant patient anxiety and may result in interventions that could interrupt a viable pregnancy. To our knowledge, no previous studies have assessed the association between the number of embryos transferred and abnormal hCG trends during very early pregnancy.

Study design, size, duration: Retrospective cohort study of 2001 successful IVF/ICSI cycles resulting in singleton live births (629 cycles with single ET and 1372 cycles with transfer of two or more embryos), between 2005 and 2014. First hCG levels were checked approximately 12 days after ET (range 7–20 days), with subsequent values checked at 2 day intervals whenever possible.

Participants/materials, setting, methods: Fresh or frozen autologous IVF/ICSI cycles with day 3 or day 5 ET were included if only one gestational sac was detected on ultrasound with a singleton live birth beyond 24 weeks of gestation. Logistic regression models were adjusted for oocyte age to estimate the Odds Ratio of having an abnormal hCG rise (<66% in 2 days).

Main results and the role of chance: The mean initial hCG values among patients receiving a single embryo or multiple embryos were 381.0 and 382.5 mIU/mL, respectively. Among patients receiving two or more embryos, 6.1% ($n = 84$) had abnormal rises between the first and second hCG measurements, compared to 2.7% ($n = 17$) of patients undergoing single ET ($p = 0.005$, OR 2.16, 95% confidence interval 1.26–3.71). Addition of the following variables to the base model did not significantly alter the association of embryo number and odds of an abnormal hCG rise: Maternal age at ET, reproductive history, infertility diagnosis, body mass index, day of ET, fresh versus frozen embryos, embryo quality, use of ICSI or assisted hatching, or endometrial thickness. Among patients with abnormal rises between the first and second hCG measurements, 89% had third hCG levels measured. In this group, normal hCG rises (>66% in 2 days) were observed between the second and third levels in 73% of patients who underwent single ET and 77% of patients who received multiple embryos.

Limitations, reasons for caution: In 20% of patients, initial hCG levels were not checked 2 days apart (range: 1–9 days); these measurements were log transformed to calculate the 2-day percent rise, in accordance with previous studies demonstrating a log-linear increase in early hCG. These findings may not be applicable to pregnancies conceived without IVF.

Wider implications of the findings: Patients with multiple embryos transferred who deliver singletons are more likely to have suboptimal early hCG rises, potentially due to transient implantation of other non-viable embryo(s). While useful for counseling, these findings should not change standard management of abnormal hCG rises following IVF. Third hCG measurements may clarify pregnancy prognosis.
Trial registration number: N/A.

P-131 Supplementation of hMG decreases early pregnancy loss in the rFSH GnRH antagonist cycles complicated with low LH levels in both young and older ages

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Study question: Whether the addition of hMG for patients complicated with low LH during COS using GnRH antagonist protocol achieves better pregnancy outcome compared to rFSH-alone in patients of different age groups.
Summary answer: Patients with low LH levels during rFSH antagonist protocols, addition of hMG reduces spontaneous abortion rates compared to rFSH-alone in both young and old ages
What is known already: The role of LH during COS remains controversial, and there has been no consistent proof that the addition of LH supplements during COS results in improvements in outcomes. Although LH deficiency has been reported in the past with varying sequelae in specific populations, it has been assumed that the LH levels in the general population receiving GnRH antagonist cycles are adequate. Our previous study have related a low cycle LH nadir with an increase in spontaneous abortion rate. It has not been shown if the addition of LH activity is associated with an improvement in outcome in young patients.
Study design, size, duration: This is a retrospective cohort comprising of 284 patients with low LH levels that were drawn from 1356 patients receiving COS using antagonist protocol between 2011 and 2014. LH levels were examined on day 2 of the cycle, 4 days after and then every 1–3 days until day of hCG. There is no loss of follow-up for the primary endpoint, which is implantation rate, pregnancy rate, and early pregnancy loss rate.
Participants/materials, setting, methods: The 1356 patients receiving GnRH antagonist protocols using rFSH or rFSH-hMG with fresh transfers were recruited. Patients with polycystic ovarian disease, hypogonadotropic hypogonadism, hyper-responders or poor responders had been excluded. The low LH is defined by a nadir ≦ 0.8 mIU/mL during COS. In the 284 patients with low LH, 174 patients received rFSH only and 110 received supplementation with hMG. Pregnancy rates, implanation rate, and spontaneous abortion rates were compared between the two groups.
Main results and the role of chance: A total of 1356 patients were recruited for analysis. We found that at least one episode of low serum LH, defined by LH ≦ 0.8 mIU/mL during COS, occurred in 284 (20.9%) patients. An analysis of the risk factors showed that age is related to the occurrence of low LH, and exhibits a decreasing trend with age (OR: 0.858, $P = 0.036$). In these 284 patients with low LH, pregnancy outcomes were compared between those receiving rFSH ($n = 174$) and those receiving rFSH-hMG ($n = 110$). The rFSH-hMG group was associated with decreased incidence of spontaneous abortions (11.5% vs. 26.7%, $P = 0.045$). The improvements in early pregnancy loss were particularly significant in younger patients (≦37 years) (4.8% vs. 20%, OR: 0.20, $P = 0.036$) and those with the occurrence of low LH before use of GnRH antagonist (3.3% Vs. 29.0%, OR: 0.08, $P = 0.012$).
Limitations, reasons for caution: The limitation of the study is in the retrospective nature of the study. Our study population is limited to the normal population and excludes polycystic ovarian disease, hypogonadotropic hypogonadism, hyper-responders and poor responders. A prospective analysis is warranted.
Wider implications of the findings: Analysis of the risk factors of low LH levels in GnRH antagonist protocol showed that younger age is related to higher occurrence of low LH. In patients receiving rFSH GnRH antagonist, it is prudent to follow LH levels to optimize cycle outcome for each patient, not limiting to older age.
Trial registration number: Nil.

P-132 Analysis of 1287 patients having unexplained spontaneous miscarriage: search for a possible etiology

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Study question: Is there any hidden etiology underlying unexplained spontaneous miscarriage (USM)?
Summary answer: Higher risk of USM in women with polycystic ovary syndrome (PCOS) is possibly due to high prevalence of hyperhomocysteinemia (HHcy) irrespective of number of miscarriages.
What is known already: Apparently-unexplained miscarriage is frustrating to the couple and perplexing to the clinicians. Moreover, the background etiology if not explored and treated at the outset, similar mishaps are likely to recur, eventually leading to well recognized clinical situation of recurrent USM. It is widely accepted that high prevalence of obesity and insulin resistance (IR) are directly related to miscarriage. A number of studies document a close association between IR and HHcy, the later being a frequent finding in our study. However, the importance of single unexplained miscarriage and the possibility of its recurrence have not been widely studied.
Study design, size, duration: A five-year prospective study including one thousand two hundred and eighty-seven women having the previous history of USM was conducted at Institute of Reproductive Medicine, Kolkata, India during November 2010–December 2015. Presence or absence of PCOS as judged by Rotterdam consensus was the initial dividing criteria while the number of miscarriage (1–2: category A; 3–4: category B, and ≥5: category C) and the presence of HHcy, IR or obesity were the basis of subsequent stratifications.
Participants/materials, setting, methods: The subjects were evaluated for uterine defects, karyotyping, thyroid disorder, HHcy, obesity, IR, thrombophilic (anticardiolipin), and infective (toxoplasma and rubella) disorders. Log-linear regression was used to determine the relative risk (RR) of USM for HHcy, IR and obesity. Student's *t*-test was used to test the difference between PCOS and non-PCOS groups while multivariate logistical regression model was used to assess the effect of homocysteine and other confounding factors like PCOS and body mass index (BMI).
Main results and the role of chance: Of the 1287 subjects, 694 had PCOS. The representation of different categories of USM in PCOS sub-population was A: 50.72% ($n = 352$), B: 36.45% ($n = 253$) and C: 12.82% ($n = 89$) as compared to A: 71.83% ($n = 426$), B: 23.10% ($n = 137$), and C: 5.06% ($n = 30$) in the non-PCOS population. The incidence of HHcy, IR and obesity was 45.67% ($n = 317$), 52.16% ($n = 362$), and 29.11% ($n = 202$), respectively, in PCOS population, which was significantly higher as compared to the non-PCOS set (HHcy: 27.65% ($n = 164$; $p < 0.04$); IR: 6.74% ($n = 40$; $p < 0.0001$); obese: 10.96% ($n = 65$; $p < 0.01$)). Hyperhomocysteinemic (RR: 0.65; 95% confidence interval (CI): 0.51–0.82), IR (RR 0.73; 95% CI: 0.51–1.02) and obese women (RR: 0.87, 95% CI: 0.74–1.03) had significantly higher chances of miscarriage. No correlation was found between miscarriage and genetic, metabolic, thrombophilic, infective and anatomical aspects. Serum homocysteine had an individual strong association with USM [odds ratio (OR): 2.01, 95% CI: 1.13–3.58) among all categories in the PCO cohort irrespective of number of miscarriages. However, using multivariate logistic regression analysis this effect was reduced to a non-significant level after adjusting for serum homocysteine [OR = 1.10, 95%CI 0.85–1.36] and all other confounding factors considered (OR = 0.98, 95% CI 0.75–1.28).
Limitations, reasons for caution: Despite including a large number of patients and using the statistical adjustment for confounding variables as homocysteine levels, PCOS, and BMI, the prospective observational analysis could not adjust for many known potential confounders, e.g., age, previous miscarriage, and infection.
Wider implications of the findings: It seems rational to diagnose the cause of the first pregnancy loss and offer treatment for better prognosis of recurrent USM. The possible involvement of HHcy, particularly in women with PCOS, may be taken into account in this direction.
Trial registration number: Not applicable.

P-133 Hyperhomocysteinemia induced soluble fms like tyrosine kinase overactivity leads to pregnancy loss in rats

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Study question: Does hyperhomocysteinemia (HHcy) influence placental thrombosis and what is the possible mechanism of action?
Summary answer: Increased soluble fms-like tyrosine kinase (sFlt-1) inhibits vasculo-endothelial growth factor (VEGF) leading to placental thrombosis and pregnancy loss in hyperhomocysteinemic rats.
What is known already: Mild-to-moderate HHcy, a risk factor for arterial and venous thrombosis, has been suggested as a possible threat to women with habitual abortions or placental abruption. Pathogenesis of vascular disease associated with homocysteine induces oxidative stress (OS) and thereby endothelial dysfunction. A hypothesis of endothelial dysfunction by excess placental secretion of sFlt-1 has recently been proposed which inhibits VEGF in the vasculature. Though HHcy, OS and thrombosis are interrelated, the directionality of that association is unclear. A better understanding of how HHcy effects pregnancy loss may help improve diagnosis in HHcy associated miscarriage.
Study design, size, duration: 40 female Sprague-Dawley rat of 5–6 week of age were mated to control males. Post pregnancy, they were divided into two groups comprising 20 female each. Pregnant control group remained sedentary. HHcy was induced in the other set by dissolving homocysteine in drinking water at a concentration of 53 mg/dL from d1 to d15 of pregnancy.
Participants/materials, setting, methods: Placental sections of homocysteine-treated and control rats were stained with hematoxylin-eosin. Circulating sFlt-1 and homocysteine concentrations were measured using commercial ELISA kits. For confirmation of OS in placenta we quantified placental heat shock protein (pHSP) by immunohistochemistry (IHC) and subsequently hypoxic inducible factor (HIF-1α) by western blot (WB). VEGF expression was evaluated by WB. Quantitative real-time PCR (qRT-PCR) was applied to determine apoptotic biomarkers in placental tissues with or without homocysteine treatment.
Main results and the role of chance: Serum homocysteine increased significantly ($p < 0.01$) in homocysteine treated cohort. Hyperhomocysteinemic rats showed reduced placental weight in comparison to control. Presence of thrombus has been observed in chorionic plate vessel with a decreased area of spongiotrophoblasts and labyrinth in the former. An increased expression of pHSP by IHC in spongiotrophoblast of experimental group and HIF-1α ($p < 0.02$) suggested fetal hypoxia and decreased oxygen supply through the placenta in HHcy. VEGF protein expression was however decreased significantly ($p < 0.01$) in hyperhomocysteinemic group. Pregnant control animals had a significantly lower level ($p < 0.0001$) of sFlt-1 (114 ± 11 pg/ml) than homocysteine treated animals (239 ± 17 pg/ml). mRNA expression of selected genes were significantly ($p < 0.01$) affected by homocysteine treatment. While survivin and Bcl-2 were found to be down-regulated, expressions of caspase 9, Bax, Bid, p21, CIDEA, HRK were up-regulated by at least threefold. Caspase-8 expression was however unaltered. This opens up a novel mechanism by which over-activity of sFlt-1 initiates the intrinsic pathway for apoptosis and fetal hypoxia, thereby inducing pregnancy loss.
Limitations, reasons for caution: Supplementary research is required to confirm this study's findings using human placental tissue. With further trials in the future in both the animal and human models, a more definitive conclusion may be arrived at.
Wider implications of the findings: A significant association between elevated homocysteine levels and pregnancy loss was observed through augmenting placental thrombosis. Over-activity of sFlt-1 may switch on OS thereby initiating the intrinsic pathway for apoptosis. Further studies of the molecular and cellular mechanisms underpinning the connection between disordered homocysteine metabolism and thrombosis are warranted.
Trial registration number: Not applicable.

P-134 Care management of patient with early recurrent miscarriages: Embryo transfer strategy

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Study question: Is the transfer at blastocyst stage a better strategy to avoid pregnancy loss and increase live birth rate in patients with recurrent miscarriages?
Summary answer: The transfer at blastocyst stage does not significantly improve live birth rate and does not significantly decrease pregnancy loss in patients with recurrent miscarriages.
What is known already: Early recurrent pregnancy loss is defined by two or more miscarriages. Different etiology are involved: immunologic, anatomic, endocrinologic, genetic disorders. When no factor is identified, the most relevant hypothesis is the production of aneuploid embryos. After embryo transfer without PGS, the miscarriage rates vary between 26% to 64%.

Embryo transfer at blastocyst stage is associated with better implantation rates and increases chances of live birth rate in general infertile populations. Extended culture leads to better embryo selection by self-correction of certain chromosomal abnormalities. Nevertheless, compared to day 3 transfer, it does not reduce miscarriage risk when euploid embryos are transferred.
Study design, size, duration: This retrospective study was conducted at our ART clinic from January 2010 up February 2015. 8681 cycles of IVF/ICSI were analyzed and couples with a history of 2 or more early miscarriages were included. The outcomes of patients who underwent fresh early cleavage embryo replacement on day 2/3, were compared to the outcomes of patients who underwent fresh blastocyst transfers (day 5/6).
Participants/materials, setting, methods: 279 (3.2%) couples fulfilled the inclusion criteria. 218 patients received an early embryo transfer and 61 a blastocyst transfer. Confounding factors, miscarriage per clinical pregnancy and live birth rate were analysed.

Clinical and biological characteristics were compared using chi-squared test and Student t-test, and IVF/ICSI outcomes were studied accordingly. Confounding factors were evaluated by a logistic regression model. The *P* value was considered significant when <0.05. The results were expressed by mean ± SD or percentage.
Main results and the role of chance: No significant differences were found between the cleavage stage transfer group and the blastocyst stage transfer group respectively in terms of female age (38.06 ± 4.04 vs. 37.65 ± 3.44), female body mass index (23.45 ± 4.38 vs. 24.03 ± 4.66), percentage of female smokers (14.36% vs. 15.09%), percentage of IVF vs. ICSI (56.88% vs. 52.46%) and the duration of ovarian stimulation.

However, less oocytes were retrieved in the cleavage stage transfer group (7.67 vs. 10.11, $p = 0.0006$) but more embryos were transferred in this group (1.94 vs. 1.70, $p = 0.009$).

No statistically significant difference of live birth rate was observed between the early transfer group and the blastocyst transfer group (8.96% vs. 16.39%, $p = 0.43$). Miscarriages per clinical pregnancy were similar in both groups (37.78% vs. 37.50%, $p = 0.69$).

After adjustment on female age, number of oocytes retrieved, fertilization technique and number of embryos transferred, a logistic regression analysis did not observe significant difference in live birth rate related to embryo transfer strategy (OR = 1.08; 95% CI: 0.87–1.34).
Limitations, reasons for caution: This study is a preliminary retrospective study, and should be confirmed by future prospective randomized studies.
Wider implications of the findings: Since PGS is not available in our country, extended culture could have been an alternative for embryo selection with reduced chromosomal abnormalities. We found that extended culture was not able to reduce miscarriages in this specific population, or to better select euploid embryos in case of underlying genetic causes.
Trial registration number: NA.

P-135 Combined first trimester risk assessment for trisomy 21 in women with recurrent pregnancy loss (RPL)

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Study question: Is the performance of combined first trimester screening for trisomy 21 different in women with RPL compared to women in the general Danish population?
Summary answer: The proportion of women, who are screen positive, is significantly higher in women with RPL compared to the background population (8.7% vs. 4.5%).
What is known already: First trimester risk assessment for trisomy 21 is based on maternal age, nuchal translucency thickness (NT) and the maternal serum markers Pregnancy Associated Plasma Protein A (PAPP-A), and free **β-hCG. In Denmark** women with a risk above 1:300 are offered an invasive diagnostic test, with a procedure related miscarriage risk of 0.5%. We hypothesized that more RPL women are screened positive in the first trimester screening based on clinical experience from a national RPL unit. This has only previously been investigated in one small study including 64 pregnancies of women with RPL – reporting no difference.
Study design, size, duration: A retrospective study based on the Danish RPL Database with RPL related data since 1986 and the Danish Fetal Medicine Database (DFMD) that covers 99.5% of the 90% of pregnancies in Denmark attending first trimester screening. DFMD includes subsequent procedures and outcomes based on data from National Birth Register, National Patient register and National Cytogenetic Register. Included are 774 singleton pregnancies of women with RPL and 268,342 population based singleton pregnancies in the period 2008–2013.
Participants/materials, setting, methods: The women included from the RPL database all have three or more pregnancy losses. Women included from the Danish Fetal Medicine Database represent 90% of Danish singleton pregnancies. Cross-linking between several of the National databases was possible after permission from the Danish Data Protecting Agency and is based on the women's unique personal registration number.
Main results and the role of chance: In total 8.7% (67/774) women with RPL were given a risk above 1:300, thus screened positive which is significantly more than the 4.5% (12,202/268,342) in the Danish population, $p < 0.0001$. The women with RPL were older (34y vs. 30y), median of PAPP-A MoM was lower (0.98 vs. 1.02), and NT was larger (1.70mm vs. 1.64mm) compared to the background population; factors resulting in higher screen positive rate. However, median of β-hCG MoM was lower (0.97 vs. 1.03) which reduce the screen positive rate.

The RPL women had significantly higher rate of fetuses with abnormal karyotypes compared to the background population (1.55% vs. 0.61%). No difference was found between the fraction of chromosomal abnormalities of the karyotyped fetuses in the RPL women 12.8% (12/94) and the Danish population 10.9% (1337/12278), $p = 0.61$.

We found higher screen positive rate and a higher proportion of fetuses with abnormal karyotypes in our cohort of RPL women. The RPL women are older, which may explain these findings. Further analyses are required to identify if the biomarkers used in the risk assessment also clinically contribute to the higher screen positive rate among RPL women.
Limitations, reasons for caution: Although this is the largest study on first trimester screening in RPL women it is still unknown if the high screen positive rate is driven alone by age or if the other biomarkers and possible other RPL related factors contribute independently.
Wider implications of the findings: It seems advantageous to inform RPL women at first trimester screening of a higher risk of being screened positive but also of chromosomal abnormalities.

More research is needed to understand the possible increase in NT and lower PAPP-A in order to individualize and optimize the first trimester screening for RPL women.
Trial registration number: The study is approved by the Danish Data Protection Agency with registration number 30-1140.

P-136 Early onset gestational diabetes (GD) as the cause for recurrent spontaneous miscarriages (RSA): RSA, pregestational metformin treatment and pregnancy outcome

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Study question: Objective of the study was to obtain a prevalence of impaired glucose tolerance, insulin resistance and early onset GD in association with RSA and analyze pregnancy outcome in this population.
Summary answer: Impaired glucose tolerance, insulin resistance show a high prevalence (98.2%) in women diagnosed with RSA. GD was diagnosed in 45.8% of women in subsequent pregnancy.
What is known already: Gestational diabetes is associated with RSA. A non diagnosed GD is a risk factor for recurrent spontaneous miscarriage. Further it is known that infertility is associates with a high prevalence of early onset GD. The efficacy of pregestational metformin treatment is supported by the observation that the rates of RSA and GD are reportedly lower among women with polycystic ovarian syndrome who conceive while taking metformin.
Study design, size, duration: Retrospective, monocentric analysis of 59 women diagnosed with RSA. Recruitment period 07/2011 to 12/2013.
Participants/materials, setting, methods: Fifty-seven women with confirmed RSA and subsequent pregnancy are included in the analysis. In all subjects a basic 75g oral glucose tolerance test (OGTT) and insulin sensitivity testing was performed prepregnancy. All RSA-patients with and without disturbed glucose metabolism or IR received metformin treatment (standard dose 3 × 500mg). As soon as pregnancy was confirmed a second 75g OGTT was performed. If impaired glucose tolerance or GD was diagnosed treatment was initiated according to the current guidelines.
Main results and the role of chance: 98.2% of the study population showed a prepregnancy disturbed glucose metabolism and/or a insulin resistance (IR). In 45.8% a GD was diagnosed in the following pregnancy. Of women diagnosed with GD 77.8% received metformin treatment prepregnancy. After initiation of metformin treatment 40 women became pregnant, all with a positive pregnancy outcome.
Limitations, reasons for caution: Retrospective Analysis, Monocentric study.
Wider implications of the findings: The high incidence of prepregnancy disturbed glucose metabolism, IR and GD indicates that glucose metabolism has more influence in miscarriage than assumed. If impaired GM/IR is not diagnosed and treated before conception or in early pregnancy, it is likely to have a negative impact on the vasculogenesis during implantation period.
Trial registration number: No conflict of interest.

P-137 Molecular karyotyping of products of conception – evaluation of performance and capacity

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Study question: Significant part of products of conception (POC) meant for investigation remain without conclusion because of classical cytogenetic testing shortcomings.
Summary answer: Chromosomal microarray analysis (CMA) is an alternative to trophoblast karyotyping capable to overcome shortcomings of the cytogenetic analysis substantially increasing investigated POC sample portion.
What is known already: Each case of miscarriage requires an in-depth investigations to determine given event etiology, pathogenesis and predict future management. About 50% of all early pregnancy loss cases are due to fetal chromosomal aberrations, which are classically analyzed cytogenetically, e.g., karyotyping which prerequisite is presence of choroidal tissues in sample. Main POC karyotyping failure reasons are tissue viability loss, inappropriate transportation conditions and time from the fetal death till testing. Molecular karyotyping – POC analysis by aCGH (array comparative genomic hybridization) can surpass cytogenetic testing because substrate for aCGH is gDNA, which can be extracted also from unviable tissue and paraffin blocks.
Study design, size, duration: Study was performed retrospectively to access applicability and effectivness of diagnostic test: molecular karyotyping of products of conception by aCGH on 37 samples received in IVF Riga clinic (Riga, Latvia) from May 2015 till January 2016. Inclusion criteria was collected (first trimester) *missed abortion* sample after medical/surgical treatment of pregnancy. aCGH for POC was validated for chromosomal aneuploydies by standart chorion villus karyotyping.
Participants/materials, setting, methods: A total of 37 POC samples were analyzed, average women age was 30.5 ± 6.3 years. In all but four investigated samples choroidal villi were clearly visualized. DNA was isolated from primary biological samples or paraffin blocks. CMA was performed following the standard protocol for aCGH (24Sure, Illumina). Resulted copy number karyotype were interpreted. To ensure that the DNA extracted from choroidal villi is free of maternal DNA three samples were STR tested.

Main results and the role of chance: Chromosomal abnormalities were found in 16 samples out of 37 (43%) (mean women age 30.5 ± 6.3). Three samples had 69, XXY karyotype, seven had autosomal trisomies by chromosomes 2, 13, 15 (2x), 16, 19 (one full and one mosaic) and 22, four were classified as X monosomies, one sample had mosaic form of monosomy by chromosome 19. Seven samples showed normal male karyotype – 46, XY and the remaining 15 exhibited balanced female aCGH profile (five of them showed no trophoblast). STR analysis test for three samples extracted from choroidal villi showed no maternal contamination in two cases but in one case low level of contamination had taken place, but its CMA profile was undoubtedly defined as chromosome 13 trisomy. POC STR analysis indicates that the DNA isolated from the visualized choroidal villi can be safely used for further analysis.

Limitations, reasons for caution: At the moment conventional POC analysis by BAC aCGH is validated for whole chromosome copy number changes. Structural chromosomal aberration (including submicroscopical) testing should be further validated. Low level mosaicism, balanced translocations and balanced polyploidies, i.e., 69,XXX and 92,XXXX cannot be detected by conventional aCGH.

Wider implications of the findings: Until now such type of testing was not available in Latvian population, but its application has substantial clinical significance. Most efficient way would be developing cost-effective strategy for POC testing, applying CMA in cases when cytogenetic testing is impossible and performing DNA contamination test in cases where no trophoblast visualized.

Trial registration number: –

P-138 Biochemical pregnancy loss is unrelated to embryo stage of development and euploidy at transfer: evidences from 2452 warming cycles

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Study question: Is biochemical pregnancy loss (BPL) decreased in cryopreserved euploid blastocyst transfers after preimplantation genetic diagnosis for comprehensive chromosomal testing (PGD-A) compared to untested cleavage-stage embryos and blastocysts?

Summary answer: Biochemical losses have similar incidence in PGD-A and untested embryo/blastocyst warming transfers suggesting no causative relationship with duration of in-vitro culture and chromosome aneuploidies.

What is known already: Biochemical pregnancy loss, defined as β-hCG levels decline after a short period of positivity, has not known underlying causes. Embryonic chromosome anomalies have been advocated as the main cause. However, no direct evidences have been reported showing that biochemical losses occur as a consequence of a primary aneuploidy in the embryo. Blastocyst stage PGD-A has been indicated as an efficient approach to accurately identify euploid embryos. The comparison of pregnancy outcomes between warming IVF cycles with or without PGD-A represents a powerful approach to directly investigate causative relationships between chromosome aneuploidies, duration of *in vitro* culture and pregnancy losses.

Study design, size, duration: A retrospective single center cohort study including all consecutive embryo transfer cycles from 2008 to 2015 was performed. Only warming cycles were analysed to avoid the potential for confounding due to hormonal imbalances deriving from ovarian stimulation. Furthermore, cleavage and blastocyst stage transfers were also evaluated separately. Three different groups were thus analysed: cleavage stage transfers without PGD-A (group A), blastocyst stage transfers without PGD-A (Group B) and blastocyst stage transfer with PGD-A (Group C).

Participants/materials, setting, methods: β-hCG was always measured on days 16th after ovulation/progesterone's administration. BPL was defined as β-hCG levels ≥10IU/L in >1occasions, but not sustained and in the absence of ultrasonographic intrauterine/extrauterine pregnancy. For group A and B, the number of embryos transferred was decided on the basis of medical and biological data, while for group C elective single euploid embryo transfers were performed. Logistic regression was used to test association between the 3groups and other cycle's/patient's covariates with BPL.

Main results and the role of chance: 2452 warming cycles involving 3241 embryos (mean per transfer = 1.32 ± 0.56) were performed in natural (74.4%) or hormone replacement cycles (25.6%) on an endometrium ≥7mm and with a level TSH ≤2.5mU/L. Mean female age at treatment was 36.25 ± 3.9, 35.4 ± 3.9, 37.4 ± 3.5 for group A, B and C, respectively (NS). 1062 (43.3%;95%CI:41.4–45.3) positive pregnancy tests were recorded, 217/637 (34.1% 95%CI:30.4–37.9; mean number of embryos transferred = 1.84 ± 0.67) following warming cleavage stage embryos, 412/1026 (40.2; 95%CI:37.1–43.2, mean number of embryos transferred = 1.3 ± 0.45) following warming blastocyst stage embryos and 433/789 (54.9%; 95%CI:51.3–58.4, mean number of embryos transferred = 1.0 ± 0.13) following euploid blastocyst transfers. Overall, 161 biochemical pregnancy losses per positive β-hCG value were observed (15.2%; 95%CI = 13.0–17.5). Biochemical pregnancy loss rate was 19.3% (42/217;95%CI = 14.3–25.2), 13.8% (57/412; 95%CI = 10.6–17.5), 14.3% (62/433; 95%CI = 11.2–18.0) for cleavage stage, blastocyst and euploid blastocyst, respectively. Logistic regression analysis showed no significant association for all covariates tested to biochemical loss incidence. In particular, blastocyst stage with PGD-A did not modify the risk (OR = 0.98; 95%CI = 0.67–1.45; $p = 0.93$) as well as the stage of transfer, cleavage or blastocyst, showed no effect (OR = 1.36; 95%CI 0.83–2.22; $p = 0.18$). Female age, male age, sperm factor, stimulation protocol, endometrial preparation protocol, coagulation factors, autoimmunity factors, were all unrelated to the risk for biochemical losses.

Limitations, reasons for caution: BPL was evaluated only from day 16 onwards. More information about the incidence of subclinical pregnancy losses and related causative factors could derive from earlier evaluations. The retrospective nature of the study is also a reason for caution. Embryo's mosaicism could not be ruled out from the analysis.

Wider implications of the findings: This study provides evidences that biochemical losses are not due to aneuploidies. Furthermore, no association with extended *in vitro* culture and other patient and cycle's characteristics were observed. Future studies looking at other genetic (non-chromosomal) and uterine factors are needed to unravel mechanisms underlying this relatively frequent adverse event reproduction.

Trial registration number: none.

P-139 Role of G-CSF treatment in recurrent pregnancy loss in TREGblood levels and expression of FOXP3, VEGF, VEGF-R2 and C-KIT in first trimester pregnancy specimens

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Study question: Which is the mechanism of action for G-CSF treatment in Recurrent Pregnancy Loss?

Summary answer: G-CSF in women with Recurrent Miscarriage increases Treg cells in the peripheral blood and in the decidua, and VEGF, VEGF-R2 and c-kit expression on trofoblast

What is known already: We used successfully G-CSF in the treatment of Recurrent Miscarriage, as well as in recurrent implantation failure. We previously showed that G-CSF may be useful in the treatment of recurrent miscarriage. The G-CSF is a cytokyne promoting leukocyte growth, but also troflobast development. However, there not data on the mechanism of action of G-CSF on pregnancy.

Study design, size, duration: An immunohistochemical and FACS studies for FoxP3, VEGF, VEGF-R2 and c-kit in first trimester placentas and blood samples of women with RPL was conducted. Tissue from 8 abortive first trimester pregnancies with RPL treated during the pregnancy with G-CSF and controls of 10 first trimester voluntary pregnancy terminations and Blood samples of 10 women with RPL treated with G-CSF and in 10 phisiological pregnancies, were collected at 7/8 week of gestation

Participants/materials, setting, methods: Immunohistochemistry was performed on tissue sections to assess FoxP3, VEGF, VEGF-R2, c-kit, with commercially available monoclonal antibodies, and nn avidin-biotin-peroxidase detection system was used.. Leukocytes extractedfrom peripheral blood samples were stained for Flow cytometric analysis using FACScalibur (BectonDickinson). All antibodies used were purchesed eBioscences. Treg cells were the CD4+CD25+Foxp3+. Statistical analysis was performed using unpaired t test.

Main results and the role of chance: In the decidua of women with recurrent pregnancy loss treated with G-CSF a significant increase in the number of cells positive to Foxp3 with respect to controls was observed (HSCORE 167+46 vs. 79+24: $P < 0.01$). VEGF expression in trophoblast of specimens of women treated with G-CSF was statistically significant higher than controls (HSCORE 156+39 vs. 70+19: $P < 0.01$) VEGF-R2 expression in trophoblast of specimens of women treated with G-CSF was statistically significant higher than controls (HSCORE 148+37 vs. 81+28: $P < 0.01$). The c-kit expression in trophoblast of specimens of women treated with G-CSF was statistically significant higher than controls (HSCORE 1417+34 vs. 72+21: $P < 0.01$) Treg levels in peripheral blood of women treated with G-CSF during pregnancy were statistically significant higher than in women with RM treated with placebo and in women with phisiological pregnancy ($P < 0.001$and $P < 0.01$ respectivelly). The other populations of lymphocytes did not show statisticaly significant differences.
Limitations, reasons for caution: Data showed that G-CSF administration in pregnant women with RPL increases the Treg cells in decidua and the VEGF, VEGF-R2 and c-kit expression on trofoblast. as well as Treg levels in peripheral blood of these women These findings show that G-CSF treatment acts on both trophoblast and maternal immune system
Wider implications of the findings: These may be the of the mechanism of action in the positive effect of G-CSF on the pregnancy outcome in these women.
Trial registration number: none.

P-140 Paternal influence of sperm transcripts and DNA damage in recurrent implantation failure

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Study question: Does sperm transcriptome affect early embryogenesis?
Summary answer: Sperm delivers selective paternal transcripts supportive of implantation and early embryogenesis to the oocyte contributing to transcriptome of embryo prior to activation of embryonic genome.
What is known already: Spermatozoa are terminally differentiated transcriptionally inactive cells, capable of post-meiotic production of functionally viable transcripts which can translate proteins involved in critical process related to stress response, embryogenesis and implantation. Sperm with DNA damage are capable of fertilizing an oocyte, but result in high rate of pre and post implantation losses. Sperm nuclear DNA fragmentation has been positively correlated with lower fertilization rates, reduced cleavage rates and rate of blastocyst development, increased embryo fragmentation and impaired implantation rates.
Study design, size, duration: A case control study of 25 men from infertile couples undergoing ART and 20 controls at AIIMS, New Delhi, India. Study duration was 9 months.
Participants/materials, setting, methods: Semen samples from men undergoing ART were analyzed for DNA damage by assessing DNA fragmentation index (DFI) and ROS (reactive oxygen species) levels (RLU/sec/million sperm) by sperm chromatin structure assay (SCSA) and Chemiluminiscence assay respectively. q-PCR analysis was performed on the semen specimens after reverse transcribing the RNA isolated from the samples. The relative quantification of target genes was calculated with $2\Delta\Delta^{Ct}$ method after normalization to β-actin.
Main results and the role of chance: The mean DFI of men undergoing ART was significantly higher (40.8 ± 5.1 vs. $22.3 \pm 6.4\%$; $P < 0.0001$) in cases as compared to controls. Seminal ROS levels were also seen to be significantly higher (452.8 ± 152.6 vs. 23.5 ± 10.7) in cases with respect to controls. The cut-off values for DFI and ROS levels were taken as <30% and <25 RLU/sec/million sperm respectively. The transcript levels of RPS6, RPL10A, RBM9, EIF5A and TOMM7 genes were correlated with DFI and ROS levels.
Limitations, reasons for caution: A potential limitation for this ongoing study is low sample size. Contributions of other confounding factors for the study namely various environmental and female factors also have the potential to influence early embryonic development and implantation.
Wider implications of the findings: The analysis of spermatozoal transcripts is important for our understanding of sperm differentiation, fertilization and early embryonic development. Correlation with oxidative stress may help in regulation of transcript levels by normalizing ROS levels. It may help to explore the diagnostic potential and assess the fecundity of spermatozoa in ART
Trial registration number: N/A.

P-141 Guideline-based quality indicators in Early Pregnancy Assessment Units

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Study question: What are valid quality indicators to measure actual care in Early Pregnancy Assessment Units (EPAUs)?
Summary answer: We identified eleven guideline-based indicators, covering four general, one patient, four logistic and two doctor aspects of care, to assess actual care in EPAUs.
What is known already: The Association of Early Pregnancy Units, Royal College of Obstetricians and Gynaecologists, the National Institute for Health and Care Excellence (UK) and the Ministry of Health, Australia have developed guidelines for setting up and running of EPAUs. A recent study showed, by using surveys, that there is considerable variation between EPAUs according to access to services and quality of care provided. To improve quality of care valid quality indicators – measurable elements of practice performance based on consensus – are needed. Quality indicators to measure the quality of care delivered by an EPAU are still lacking.
Study design, size, duration: We used the systematic RAND-modified Delphi method to develop an indicator set from these four available guidelines. This stepwise method consists of four expert consensus rounds with independent expert ratings and repetitive feedback. The expert panel comprised a representative diversity of 11 experts in the field and clinical guideline users working at academic and teaching hospitals in the UK, Australia, Denmark and The Netherlands.
Participants/materials, setting, methods: We divided the recommendations extracted from the guidelines into four domains, i.e., general, patient, logistic and doctor, and translated these into a questionnaire presented to the expert panel. Every member scored each recommendation on a nine-point Likert scale. The panel prioritized the recommendations using a top-five ranking system per domain. We selected those >75th percentile as key recommendations, A second questionnaire with the member's scores presented the ranking of recommendations.
Main results and the role of chance: We extracted 119 recommendations from the guidelines of which 37 general, 13 patient, 51 logistic and 18 doctor aspects of care. Ten out of 11 questionnaires (91%) were returned by the expert panel and fully completed. The highly scored recommendations were selected for the set of potential key recommendations. These key recommendations are: (1) available protocols for daily practice, (2) all treatment options available for miscarriage, (3) all treatment options available for ectopic pregnancy, (4) a standard system to register ultrasound findings, (5) good quality ultrasound equipment, (6) access to urine pregnancy testing, (7) access to serum hCG assay, (8) a designated examination room to provide privacy, (9) a designated senior staff member responsible for the clinical area, (10) recognized ultrasound training for the staff and (11) a system taking care that women referred to an EPAU are seen promptly. No new recommendations were suggested by the expert panel.
Limitations, reasons for caution: A limitation can be the selection of the expert panel. This can influence the accuracy and validity of the procedure. On the other hand, there is no good alternative for this.
Wider implications of the findings: Guideline-based quality indicators can help clinicians to establish and run an high quality evidenced based EPAU. Furthermore, indicators enable the measurement of actual care and guideline adherence in and between various EPAUs. Further research can focus on barriers and facilitators for guideline dissemination to further optimise the quality of care.
Trial registration number: Not applicable.

P-142 Maternal & perinatal outcome of pregnancies threatening to miscarry

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Study question: The purpose is to study maternal and perinatal outcome in women with threatened miscarriage**.**

Summary answer: There is need to monitor patients after a threatened miscarriage to minimise the complications and, such pregnancies demand more serious prenatal care.
What is known already: These pregnancies are at a higher risk but there is no definitive evidence.
Study design, size, duration: RCT: A large, prospective, multiinstituional study was conducted over a period of 36 months in two tertiary care centers in Northern India.746 Pregnant patients with a history of threatened miscarriage during first twenty weeks of pregnancy. They were registered, followed at antenatal clinics and delivered. Ultrasonogram was performed for diagnosis, calculation of gestational age and to detect the presence of subchorionic hepatoma. Controls, without a history of threatened miscarriage were similarly scanned and followed throughout pregnancy.
Participants/materials, setting, methods: The patients registered, followed up prospectively at antenatal clinics and delivered in same hospital. Ultrasonogram was performed for diagnosis, calculation of gestational age and to detect the presence of subchorionic hematoma. The patients were followed up regularly. In patients with subchorionic hematoma, scans were repeated weekly until resolution of hematoma. All women (controls and cases) were matched for age, parity, social class, BMI and gestational age at booking.
Main results and the role of chance: The overall adverse pregnancy outcomes were significantly higher in women with threatened miscarriage than the control group. Out of 746 cases, 179 (24%) patients spontaneously aborted after diagnosis of threatened miscarriage. Low lying placenta on USG at the initial scanning before 20 weeks was significantly more in study group than control group ($p = 0.02$). But at term the difference was not significant which can partially be explained by theory of trophotropism of placenta.

The incidence of preterm labor, PROM, low birthweight was significantly higher among the cases amd more in patients with heavy bleed. The mean birthweight and mean gestational age at birth was significantly less among females with threatened miscarriage. A high percentage of patients had subchorionic hemorrhage on early USG in the study group while none in the control group had it. The incidence of spontaneous miscarriage was significantly higher in patients with hematoma ($p = 0.01$). This was also dependent on the size of hematoma.
Limitations, reasons for caution: Subjectivity in amount of bleeding and USG interpretation.
Wider implications of the findings: The identification of these high risk groups should enable better management protocols and new therapeutic protocols to improve neonatal outcome. The most encouraging aspects of our study were that it was a prospective study, women were booked very early in pregnancy, multiple fetal as well as maternal outcomes were studied.
Trial registration number: Not applicable.

POSTER VIEWING SESSION

EMBRYOLOGY

P-143 Blastocyst collapse is not an independent predictor of reduced live birth: a time-lapse study

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Study question: Is blastocyst collapse, observed by time-lapse monitoring (TLM), independently associated with live birth in a cohort of unselected infertile patients undergoing single blastocyst transfer?
Summary answer: Blastocyst collapses (including multiple ones) occurred frequently in almost half of the cohort, but they were not independent predictors of reduced live birth.
What is known already: In a recently published retrospective study (Marcos et al., 2015), (mostly single) blastocyst collapse, precisely observed by TLM, was associated with a reduced rate of implantation (35 versus 48.5%). The authors strongly recommended against transferring such blastocysts (if any alternative is available) and encouraged to include this marker as an additional criterion in existing time-lapse embryo selection models.
Study design, size, duration: All consecutive infertile patients whose embryos were submitted to prolonged embryo culture and reached expanded blastocyst stage ($n = 501$) in a TLM incubator (EmbryoScope, Vitrolife) between 2012 and 2014, subsequently undergoing vitrified-warmed embryo transfers ($n = 291$) until August 2015 were included in this retrospective analysis. Fourteen cases involving zona pellucida-free embryos were excluded because blastocyst collapse could not be evaluated in them. The outcome of embryo transfers was followed-up until live birth occurred (including 4 ongoing pregnancies).
Participants/materials, setting, methods: Early, late and interval morphokinetic parameters were scored according to published consensus criteria. Blastocyst collapses were defined as instances when the surface of the trophoectoderm cells separated >50% from the inner side of the zona pellucida. The entire cohort was divided into three subgroups: "no collapse", "single collapse" or "multiple collapses". The association between blastocyst collapse and live birth was evaluated by a multivariate logistic regression analysis including categorical morphokinetic variables and other confounders.
Main results and the role of chance: During the study period 277 single blastocyst transfers were performed (female age 38.4 ± 3.9, range: 28–47 years). The overall live birth rate per embryo transfer was 30% (82/277). One or more blastocyst collapse(s) occurred in 46% (127/277) of the examined embryos; in 22% (61/277) of the cases only a "single collapse" was observed whereas in 24% (66/277) "multiple collapses" were seen. In the "multiple collapse" subgroup an average number of 2.9 collapses were registered (range: 2–9). Live birth rates per single blastocyst transfer decreased progressively between subgroups (36, 31 and 14%, respectively, $p = 0.004$) but statistically significant differences were only observed between the "multiple collapse" and the other two subgroups ($p = 0.0009$ and 0.02). Unadjusted odds ratios showed that only "multiple collapse" was associated with a decreased probability of live birth (OR: 0.28 95%CI: 0.12–0.59, $p = 0.001$). After adjusting for 8 categorical TLM variables and 7 patient-, and cycle-related confounders the previously seen association for the "multiple collapse" subgroup has disappeared. In the resulting model only $texpB_2$ (time to reach expanded blastocyst size of 160 microns) (aOR 4.67 95%CI: 1.62–14.9, $p = 0.006$), t2 (aOR 2.44 95%CI: 1.27–4.78, $p = 0.008$) and female age (aOR 0.87 95%CI: 0.79–0.96, $p = 0.006$) remained significantly associated with live birth.
Limitations, reasons for caution: The main limitation was the moderate number of 277 blastocyst transfers included. Furthermore our unselected cohort was biased towards advanced-aged, poor-prognosis patients undergoing mild IVF treatment coupled with vitrified-warmed single blastocyst transfer exclusively. This might limit generalizibility to other less infertile populations or to centres which use different treatment protocols.
Wider implications of the findings: Blastocyst collapse patterns should not be evaluated alone without taking into account other cleavage- or blastocyst-stage morphokinetic variables (such as $texpB_2$ and t2) that are stronger predictors of reproductive outcome.
Trial registration number: n/a.

P-144 A hierarchical model combining cleavage-stage morphokinetic variables predicts blastocyst formation rate

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Study question: Which cleavage-stage morphokinetic variables are the most predictive of expanded blastocyst formation rate in order to establish a hierarchical model based on the strongest predictors?
Summary answer: In a multivariate analysis three cleavage-stage variables (t2, t9, and s3) were associated with blastocyst formation permitting the establishment of a simple, combined hierarchical score.
What is known already: In recent years increasing number of time-lapse monitoring (TLM) studies focused on finding morphokinetic variables that could be predictors of blastocyst formation, succesfully identifying several (early) cleavage-stage variables that were consistently associated with it. However, only a recent study (Motato et al., 2015) has managed to estabilish a four-category hierarchical score (including tM = time to morula and s3 = t8–t5) predicting blastocyst development which showed a good discrimination ability (AUC: 0.849). It is arguable however, whether including tM is really useful if a blastocyst prediction model would be used in a clinical setting to select day 3 embryos for prolonged culture.

Study design, size, duration: All consecutive infertile patients from a single centre whose cleavage-stage embryos were submitted to prolonged culture (n = 1137) in a TLM incubator (EmbryoScope, Vitrolife) between 2012 and 2014 were included in this retrospective analysis.
Participants/materials, setting, methods: Early (PNf, t2–9) and interval (cc2a-b, s2–3) morphokinetic parameters were scored according to consensus criteria and standardized to pronuclear fading. Detailed histograms for all morphokinetic variables were examined to determine the existence of ranges with high, medium and low rates of blastocyst development. Subsequently continuous TLM variables were converted into categorical ones. The association between blastocyst formation and categorical morphokinetical variables was evaluated by multivariate logistic regression analysis also including other patient-, and cycle-related confounders.
Main results and the role of chance: The average rate of expanded blastocyst formation was 44% (501/1137). In a univariate analysis all twelve cleavage-stage morphokinetic variables and also eight patient- (female age, parity, history of previous IVF treatment, partner age, duration of marriage as a proxy for infertility duration), and cycle-related confounders (stimulation type, current cycle rank, number of retrieved mature oocytes) were associated with blastocyst formation. In a multivariate analysis however, only three cleavage-stage (t2, t9 and s3) and two patient-related confounders (female age and duration of marriage) remained statistically significant. Combining the two strongest morphokinetic predictors (t2 and s3) resulted in a simple, five-category (from "E" to "A") hierarchical model, representing 16, 17, 23, 28, and 16% of the entire cohort of cultured embryos, respectively. The model effectively distinguished between embryos of increasing blastocyst development potential (11, 23, 43, 62 and 72% from category "E" through "A", $p < 0.0001$). The AUC value of the hierarchical model was higher (0.744) than for any of the two combined cleavage-stage variables alone (0.669 for t2 and 0.696 for s3).
Limitations, reasons for caution: Our unselected cohort was biased towards advanced-aged, poor-prognosis patients undergoing mild IVF treatment coupled with single vitrified-warmed blastocyst transfer. This might limit generalizibility to other less infertile populations or to centres which use different treatment protocols.
Wider implications of the findings: Our study suggests that both early (t2) and late (s3 as t8–t5) cleavage-stage morphokinetic parameters are associated with development until the expanded blastocyst stage. A simple, hierarchical model effectively distinguished between day 3 cleavage-stage embryos of low, intermediate and high blastocyst development potential permitting their ranking before starting prolonged culture.
Trial registration number: None.

P-145 What are pregnancy chances after the transfer of day 5 vitrified/warmed compacted morulæ and early blastocysts as compared to late blastocyst?

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Study question: Is it worth cryopreserving compacted morula and early blastocyst stage embryos?
Summary answer: Compacted morula, early blastocyst and late blastocyst stage embryos show equal transfer rate, implantation rate and pregnancy rate after warming.
What is known already: The excellent results reported on embryo vitrification at day 5 were based on the selection of top embryos which implies that lower quality embryos are ruled out from transfer. However it is of common knowledge that Day 5 embryo cohorts obtained after *In vitro* culture often show heterogeneous embryo stages ranging from compacted morula to late blastocysts based. These dissimilarities reflect individual embryo dynamics that provide different success chances. Day 5 embryos displaying typical morphology may be cryopreserved. However, the prognosis in terms of transfer, implantation and pregnancy rates for each embryo grade has not yet been investigated.
Study design, size, duration: 543 evolving embryos obtained at day 5 were vitrified over a four-year period: Category 1, either compacted morula or blastocyst with early forming blastocoele (n = 257), Category 2, early blastocyst with clear blastocoele that occupies less than half of the embryonic volume (n = 233) and Category 3, late blastocysts displaying a blastocoele occupying more than half the volume of the embryo and/or increased size (n = 53). Transfer, implantation and pregnancy rates were assessed and compared between each category.
Participants/materials, setting, methods: The 543 embryos were vitrified either for delayed embryo transfer (freeze-all strategy) or for the preservation of surpernumerary embryo. Embryos were individually cryopreserved (Cryotop®) and warmed using the Kitazato vitrification/warming kit. Morphology was assessed using the simplified Gardner grading system and recorded prospectively in an image database before vitrification and after warming. Transfer, implantation and pregnancy rates were assessed for each embryo category and compared using SPSS (Statistical Package for the Social Sciences) software.
Main results and the role of chance: 404 couples enrolled in infertility treatment program benefitted embryo vitrification at day 5. The main objective of the study was to assess and to compare post-warming embryo transfer, implantation and pregnancy rates in the 3 different day 5 embryo categories obtained after *In Vitro* Fertilization, Intra Cytoplasmic sperm injection using fresh or vitrified/warmed oocytes. The major outcome of the present investigation is that, after warming, day 5 embryos belonging to the 3 categories described above show equal success rates: category 1, 2 and 3 embryos have comparable chances to be transferred after warming (88.3%, 88.3%, 93.0%, respectively, [p = 0.55]), they have similar implantation rates (n fetal sac/n embryo transferred) (15.8%, 14.4%, 26.3%, respectively, [p = 0.21]) and provide equivalent early pregnancy chances (n clinical pregnancy/n transfer) (16.3%, 15.9%, 26.4%, respectively [p = 0.17]). Miscarriage rate encountered after embryo transfer is statistically not different in the 3 categories (9.1%, 15.8%, 6.3%, respectively, [p = 0.12]). Our data show that the technique used for fertilization does not affect embryo post-warming transfer rate (p = 0.99), implantation rate (p = 0.65), clinical pregnancy rate (p = 0.65) nor miscarriage rate (p = 0.56).
Limitations, reasons for caution: Category 3 embryos are commonly transferred in priority during fresh cycle. Therefore, the number of vitrified embryos belonging to this category is much smaller than others leading to a loss of power of statistical analyses. Larger group of category 3 embryos is required to draw final conclusion.
Wider implications of the findings: Our observation shows that as long as an evolving embryo reaches day 5 and displays typical morphology, it constitutes a chance to achieve a pregnancy. Therefore, it is worth cryopreserving pre-blastocyst and early blastocyst. We believe that interest should be borne at such embryos that are unfortunately often discarded.
Trial registration number: None.

P-146 Algorithms for the prediction of blastocyst formation and implantation: the predictive value of oocyte and cleavage-stage embryo morphological criteria

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Study question: Are there algorithms, based on oocyte and embryo morphological criteria assessed under light microscopy, which can predict blastocyst achievement and implantation potential in ICSI cycles?
Summary answer: This study characterized variables with independent predictive value and provided algorithms for the prediction of blastocyst formation and implantation potential in ICSI cycles.
What is known already: A prospective and individual determination of whether an extended embryo culture strategy would improve the chance of pregnancy is challenging. This challenge becomes more complicated when we consider our inability to predict whether (or which) cleavage stage embryos will form viable blastocysts. In order to define cycles that would benefit from extended embryo culture and result in higher chances of implantation, it is important to determine (i) which cleavage-stage morphological criteria would be able to predict blastocyst formation, and (ii) which of these criteria would be able to assist in blastocyst selection for transfer.

Study design, size, duration: This transversal study included data from 743 ICSI cycles performed from July 2011 to June 2014 in a private fertility centre. Inclusion criteria were as follows: patients undergoing ICSI with fresh embryo transfer performed on day 5 of development. A total of 5850 individually cultured embryos were evaluated regarding independently identified oocyte dimorphisms, pronuclear score and embryo morphological scores on days 2 and 3.
Participants/materials, setting, methods: Associations between these variables and the probability of the achievement of a blastocyst or of an ongoing implantation were checked and candidate predictors were considered for the construction of a multivariable prediction model to rank embryos according to their blastocyst formation and implantation potential. The characteristics of the cycle, couple and treatment were also included in the model.
Main results and the role of chance: Based on the bivariate analysis, 43 potential predictive variables for blastocyst formation potential and 20 for blastocyst implantation potential were selected for the establishment of prediction models using binary multiple logistic regression. The final prediction model included 14 independent predictive factors for blastocyst formation: Female age, oocyte yield, large perivitelline space in the oocyte, morphological features on day 2 (cleavage rate, blastomere asymmetry, blastomere multinucleation, multinucleation in more than 25% of the blastomeres, and absence of a nucleus in at least one blastomeres), morphological features on day 3 (number of blastomeres, cleavage rate, blastomere asymmetry, fragmentation, blastomere multinucleation, and the number of blastomeres with no apparent nucleus). The final prediction model included four independent predictive factors for the blastocyst implantation potential model: female age and morphological features on day 2 (blastomere asymmetry, multinucleation, and absence of a nucleus in at least one blastomere). The prediction models for blastocyst formation potential and implantation potential achieved accuracy rates of 71.7% and 80.1%, respectively.
Limitations, reasons for caution: In most laboratories, embryo selection for transfer is still based on morphological parameters evaluated by light microscopy. The subjectivity of the morphological evaluation, as well as the wide diversity of embryo classification systems used by different centers implies contrasting results, making the implementation of consensus a difficult task.
Wider implications of the findings: These models provide a tool for clinics and embryologists that predicts the best time for embryo transfer without the use of time-lapse technology. The optimization of embryo selection, considering morphological criteria from early stages, represents the potential to increase treatment success rates while minimizing the chances of a multiple pregnancy.
Trial registration number: N/A.

P-147 A simple, hierarchical model combining cleavage- and blastocyst-stage time-lapse variables successfully predicts live birth following vitrified-warmed single embryo transfer

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Study question: Which early/late morphokinetic time-lapse variables are the most predictive of live birth in order to establish a hierarchical model based on the strongest predictors?
Summary answer: In a multivariate analysis one cleavage- and one blastocyst-stage morphokinetic variable was associated with live birth permitting the establishment of a simple, combined hierarchical score.
What is known already: In recent years increasing number of time-lapse monitoring (TLM) studies focused on finding morphokinetic variables that could be predictors of embryo implantation. Most of these studies involved cleavage-stage embryos only (between 84 to 754 "KID" embryos with so-called known implantation data) and succesfully identified several early cleavage-stage morphokinetic variables (such as t3, t5, cc2, s2 and cc3) that were consistently associated with successful implantation. On the other hand, so far there is a lack of published studies involving time-lapse monitored blastocysts and morphokinetic parameters extracted from later stages (morula, early and expanded blastocyst) of embryo development are still under investigation.
Study design, size, duration: All consecutive infertile patients from a single centre whose embryos were submitted to prolonged embryo culture and reached the expanded blastocyst stage (n = 501) in a TLM incubator (EmbryoScope, Vitrolife) between 2012 and 2014, subsequently undergoing vitrified-warmed embryo transfers (n = 291) until August 2015 were included in this retrospective analysis. The outcome of embryo transfers was followed-up until live birth occurred (n = 86) (including 4 still ongoing pregnancies).
Participants/materials, setting, methods: Early, late and interval morphokinetic parameters were scored according to published consensus criteria and standardized to pronuclear fading. Subsequently, histograms for all morphokinetic variables were examined to determine the existence of "optimal live birth ranges". Those TLM variables where such an optimal LB range could be observed were converted into categorical ones. The association between live birth and categorical morphokinetical variables was evaluated by multivariate logistic regression analysis including other patient-, and cycle-related confounders.
Main results and the role of chance: The examination of detailed histograms revealed that areas with a significantly higher proportion of live births ("optimal LB ranges") existed for five cleavage-stage (t2, t3, t4, cc2a and cc2b) and three blastocyst-stage variables (tBfull, texpB_1 and $_2$) thus permitting the conversion of these morphokinetic variables from continuous (hours) to categorical (inside/outside) ones. After adjusting all eight morphokinetic parameters and seven patient- and cycle-related confounders among each other, only two TLM variables remained highly significant (t2: aOR 2.42 95%CI: 1.29–4.57, p = 0.006 and texpB_2: 4.37 95%CI: 1.62–12.94, p = 0.005). Combining these two strongest predictors resulted in a simple, three-category ("A", "B" and "C") hierarchical model. Category "A", "B" and "C" blastocysts represented 22, 49 and 29% of the total cohort, respectively. The model effectively distinguished between blastocysts of increasing live birth potential (category "C": 11%, category "B": 30%, category "A": 54%, $p < 0.0001$). Compared to the middle category "B" group, "A" and "C" blastocysts had a significantly higher (OR 2.72 95%CI: 1.47–5.10, p = 0.00015) and lower (OR 0.25 95%CI: 0.10–0.55, p = 0.00009) LB potential, respectively. The AUC value of the hierarchical model was higher (0.70) than for any of the two combined variables alone (0.61 for t2 and 0.66 for texpB_2).
Limitations, reasons for caution: The main limitation is the moderate number of 291 single blastocyst transfers included. Furthermore our unselected cohort was biased towards advanced-aged, poor-prognosis patients undergoing mild IVF treatment coupled with single blastocyst transfer exclusively. This might limit generalizibility to other less infertile populations or to centres which use different treatment protocols.
Wider implications of the findings: Our study suggests that blastocyst-stage morphokinetic parameters might be better predictors of clinical outcome than previously published cleavage-stage ones (higher odds ratios for texpB_2 compared to t2). A simple, hierarchical model effectively distinguishes between low, medium and high implantation potential blastocysts permitting their ranking before future embryo transfers.
Trial registration number: n/a.

P-148 Oocytes with smooth endoplasmic reticulum clusters originate blastocysts with impaired implantation potential

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Study question: Are embryos derived from oocytes presenting smooth endoplasmic reticulum clusters (SERc) less likely to develop into blastocysts and implant?
Summary answer: In this study, although oocytes displaying SERc normally reached the blastocyst stage, no blastocysts derived from SERc+ oocytes implanted.
What is known already: The Alpha Scientists in Reproductive Medicine and ESHRE Special Interest Group of Embryology recently recommended not inseminating oocytes affected by SERc, since they might be associated with an increased risk of abnormal outcome. Several reports focusing specifically on SERc+ oocytes have indeed shown negative outcomes in terms of fertilization, embryo development and pregnancy rates as well as compromised obstetric and neonatal outcomes. However, a more recent publication demonstrated that healthy babies could result from SERc+ oocytes. Therefore, information regarding the clinical significance of SERc+ oocytes is still controversial.

Study design, size, duration: This transversal study included data from patients undergoing their first or second ICSI cycle, from July 2011 to June 2014, in a private fertility centre. Inclusion criteria were as follows: patients undergoing ICSI with fresh embryo transfer performed on day 5 of development.
Participants/materials, setting, methods: The obtained oocytes were split between the SERc+ group, which consisted of oocytes presenting SERc, and the SERc-group, which consisted of oocytes free of SERc. The ICSI cycle outcomes were compared between the SERc+ and SERc-groups. Additionally, the implantation rate was compared between the groups. For the investigation of implantation, only cycles in which none (0%) or all the embryos transferred had implanted (100%) were included in the analysis.
Main results and the role of chance: Seven hundred and forty-three ICSI cycles performed were analyzed. A total of 7609 oocytes were morphologically evaluated and 167 oocytes presented SERc. Maternal age and the total dose of FSH administered were not associated with the occurrence of SERc. However, the number of follicles and retrieved oocytes were determinants of the increased odds of SERc occurrence (OR: 1.03; CI: 1.02–1.04 and OR: 1.03; CI: 1.01–1.04, respectively). A total of 5705 zygotes were obtained. There were no significant differences between the SERc+ and SERc-groups regarding the fertilization rate and high-quality embryo rate on days 2 and 3. On day 5 of embryo development, 3147 blastocysts were obtained. Similar blastocyst formation rates and number of transferred embryos were observed between the groups. Out of 1639 embryos transferred, 767 blastocysts were transferred to patients who had a 0% or 100% implantation rate. A total of 618 blastocysts were transferred in the 0% implantation rate group and 149 blastocysts were transferred in the 100% implantation group. The mean implantation rate in the SERc-group was 20.5% whereas no blastocyst derived from SERc+ oocytes implanted.
Limitations, reasons for caution: Approximately 5% of ICSI cycles show at least one SERc+ oocyte. The relatively rare occurrence of SERc in oocyte cohorts limits the experimental analysis of this dysmorphism. Additionally, a bias might have been introduced since oocyte morphology in this study was punctually accessed by light microscopy.
Wider implications of the findings: The birth of healthy babies from SERc+ oocytes might lead to a revision of the current consensus in the future. In the meanwhile, if transfers of embryos derived from SERc+ oocytes are performed, they should be approached with caution and only when no alternative embryos of sufficient quality are available.
Trial registration number: N/A.

P-149 Freeze-all, oocyte vitrification or fresh embryo transfer? Lessons from an egg-sharing donation program

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Study question: Which approach leads to better results, embryo vitrification, oocyte vitrification, or fresh embryo transfer in controlled ovarian stimulation (COS) cycles?
Summary answer: Better clinical outcomes result when using embryo vitrification, followed by oocyte vitrification. Fresh embryo transfer in COS cycles leads to poorer results.
What is known already: The cryopreservation of oocytes is a popular technique that is useful in oocyte donation. It has been reported that COS is associated with impaired endometrial receptivity. In frozen–thawed cycles, when COS is not performed, pregnancy rates have been reported to be higher than in fresh cycles. Considering this, the freeze-all policy has emerged as an alternative to fresh embryo transfers. However, while it has been suggested that embryo transfer in natural or endometrium-prepared cycles is a better approach than the transfer during a COS cycle, the question about vitrification of oocytes or embryos remains under debate.
Study design, size, duration: This case-control study included 8,075 oocytes from 425 oocyte donors that were submitted to intracytoplasmic sperm injection (ICSI), from Jan/2014 to Dec/2014. The oocytes were used for the donors' own cycles (n = 5440/4585 oocytes/embryos, the Fresh_Oocytes_Group) or were cryobanked (n = 2635/2128 oocytes/embryos, the Vitrified_Oocytes_Group) for 425 recipients. Concerning the Fresh_Oocytes_Group, the embryos were either cryopreserved and transferred during a subsequent cycle (Freeze_all_Group, n = 297 cycles, 3209 embryos) or transferred during a fresh cycle (Fresh_Transfer_Group, n = 128 cycles, 1307 embryos).
Participants/materials, setting, methods: The study was performed at a private assisted reproduction centre and enrolled oocyte donor patients undergoing ICSI cycles using fresh oocytes, vitrified oocytes, or vitrified embryos. Mature oocytes were vitrified three hours after collection and cryo-stored. Embryos were vitrified on day three of development. Both the vitrification and warming procedures were performed using the Cryotop method. On day five, for both the fresh and cryopreservation protocols, one or two embryos were transferred.
Main results and the role of chance: The fertilisation rate (85.4 ± 14.4 vs. 80.2 ± 18.2, $p < 0.001$) and number of transferred embryos (1.6 ± 1.0 vs. 1.8 ± 1.1, $p < 0.001$) was lower for the Vitrified_Oocytes_Group compared to the Fresh_Oocytes_Group. In addition, the high quality embryo rate on days two (43.2% vs. 31.5%, $p < 0.001$) and three (38.6% vs. 30.7%, $p < 0.001$) and the blastocyst formation rate (41.1% vs. 36.6%, $p < 0.001$) were lower for embryos derived from vitrified oocytes compared to embryos derived from fresh oocytes. When the Fresh_Oocytes_Group was split into the Freeze_all_Group and the Fresh_Transfer_Group and these groups were compared with the Vitrified_Oocytes_Group, a significant difference in the clinical outcomes was noted: the Freeze_all_Group had the highest pregnancy (71.4% vs. 49.6% vs. 39.8%, $p < 0.001$) and implantation rates (67.3% vs. 43.0% vs. 37.2 $p < 0.001$), followed by the Vitrified_Oocytes_Group, while the Fresh_Transfer_Group had the lowest rates of pregnancy and implantation. No significant differences were noted when miscarriage rates were compared among the groups (10.8% vs. 12.8% vs. 9.4%, $p = 0.679$).
Limitations, reasons for caution: For the present study, the oocytes were obtained from an egg-sharing donation program; therefore, they originated from infertile couples. Moreover, the limited number of subjects may be a reason for caution in the present study.
Wider implications of the findings: Embryo vitrification is a better approach than oocyte vitrification. However, when compared to fresh embryo transfers, embryos derived from warmed oocytes and transferred into a "more receptive endometrium" result in better clinical outcomes. This supports the value of oocyte vitrification for cryobanking in oocyte donation programs.
Trial registration number: N/A.

P-150 Treatment with ionomycin improves pregnancy rates in poor responders

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Study question: Does ionomycin treatment improve embryo developmental competence and pregnancy rate of poor responders?
Summary answer: Application of ionomycin treatment leads to increased rates of cleavage to 8-cell stage and clinical pregnancy.
What is known already: Ionomycin can induce Ca^{2+} influx by altering the plasma membrane permeability or can act directly on intracellular organelles which release Ca^{2+}. Studies in somatic cell nuclear transfer bovine oocytes showed that ionomycin is a more potent and more specific Ca^{2+} ionophore than A23187. When ionomycin is used as an assisted oocyte activation agent, the reported fertilization rates are usually higher than with the use of A23187. In humans, calcium fluctuations were detected with a peak shortly before cell division. Interestingly, these calcium oscillations disappeared in arrested embryos.
Study design, size, duration: This prospective study was performed between October 2014 and December 2015 at a reproductive center. All patients involved gave written consent, and institutional review board approval was granted. This study includes 21 non-treatment and 22 treatment couples.
Participants/materials, setting, methods: A prospective study was conducted including patients with a poor responders following conventional ICSI in our center. In the treatment cycles, all metaphase II-oocytes were exposed to a commercially available ready-to-use 0.5 μM ionomycin for 5 min immediately after ICSI. After a three-step washing procedure, *in vitro* culture was performed as in the control cycles, when possible up to 8-cell stage. Cleavage and clinical

pregnancy rates were compared, *p*-value of <0.05 was considered statistically significant.

Main results and the role of chance: The average female age, number of oocytes retrieved, embryo developmental stage and number of embryos transferred did not vary significantly amongst the groups. Fertilization rate did not differ (75.4 versus 73.2%); however, further cleavage to 8-cell stage was significantly higher ($P < 0.001$) in the ionomycin group (98.5%) when compared with the control cycles (91.9%). Most importantly, clinical pregnancy rates were higher ($P < 0.01$) in the ionomycin treatment group (44.4%) when compared to the control group (20.8%).

Limitations, reasons for caution: Poor responders exhibit advanced age, previous ovarian surgery or pelvic adhesions, all of which can contribute to reduced oocytes and embryo quality in comparison with normal responders. This study is still not powered to exclude possible associations between take home baby rate and perinatal outcomes on babies born.

Wider implications of the findings: The findings of this study indicate that ionomycin treatment improves the rates of cleavage to 8-cell stage and clinical pregnancy in poor responders. However, this treatment does not seem to completely resolve the poor responders. Further investigations are necessary to determine the effects of ionomycin treatment of the culture conditions.

Trial registration number: NA.

P-151 Prostaglandin-Endoperoxide Synthase 2 and Versican expression levels in cumulus cells are associated with *in vitro* fertilization pregnancy outcomes

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Study question: Is expression of Prostaglandin-Endoperoxide synthase 2 (PTGS2) and Versican (VCAN) in Cumulus cells' (CC) correlated with embryo pregnancy potential?

Summary answer: Is expression of Prostaglandin-Endoperoxide synthase 2 (PTGS2) and Versican (VCAN) in Cumulus cells' (CC) correlated with embryo pregnancy potential?

What is known already: Many reports have established the association between CCs gene expression profiles and oocyte competence, embryo quality, and pregnancy outcomes. For example, PTGS2 expression was associated with germinal vesicle to metaphase II stage transitions, development of higher quality embryos, and good embryo morphology. Moreover, VCAN, a major component of the extracellular matrix of the cumulus-oocyte complex, has been associated with oocyte quality, key steps of oocyte maturity, embryonic development, cumulus expansion, and higher probability of pregnancy and live births, making PTGS2 and VCAN gene expression plausible markers of ideal oocytes for IVF procedures.

Study design, size, duration: A retrospective study design was utilized for one complete IVF cycle. One hundred and ninety-eight women that suffer from infertility undergoing IVF in Mexico City, Mexico were selected for this study. Some subjects were lost due to not returning for follow-ups appointments, failure to produce viable oocytes/embryos, failure to collect sufficient RNA from the CCs for analysis, or chose not to be included. Forty-two women healthy were included (age: 29–46 years; BMI = 25.5 ± 5.0 kg/m^2).

Participants/materials, setting, methods: Patients were subjected to a controlled ovarian stimulation protocol with GnRH. CCs were isolated during oocyte retrieval and RNA was isolated using Trizol per the manufacture's suggestions. The expression of PTGS2, VCAN, and L19 were measured by using quantitative PCR. The PVL index [$(PTGS^{2+}VCAN)*L19_{normalized}$] was determined for each oocyte. After fertilization, only embryos with high morphological scores were implanted. Clinical pregnancy was confirmed by βhGC or the presence of a fetal heartbeat.

Main results and the role of chance: 8.2 ± 3.4 oocytes were collected per patient, of which 47.7 ± 29.5% of the collected oocytes had PVL scores ≥58. There was no correlation between PVL index and morphological scores. 2.3 ± 0.8 embryos were implanted per a patient. Using a modified equation proposed by Ekart et al., we calculated the probability of pregnancy for each treatment. The presence of at least one embryo with a PVL index score was associated with a greater chance of achieving clinical pregnancy (odds ratio=5.464, 95%CI: 1.01–29.54). However, 13 subjects presented endometriosis and, when excluded, the chance associated with achieving clinical pregnancy increased (odds ratio=14.00, 95%CI: 1.42–137.32).

Limitations, reasons for caution: Due to regulations in Mexico, only few women received single embryo transfers. A majority of women received three embryos; however, the number of embryos with PVL index scores of ≥58 ranged from one to three per cycle. We cannot be sure which embryo led to a clinical pregnancy.

Wider implications of the findings: Here, we provide evidence that using embryos with PVL index scores ≥58 could improve IVF outcomes. This result was found in Mexicans; however, it is possible the index can be implemented in other ethnicities.

Trial registration number: None.

P-152 Ultrastructure and cytogenetic analysis of bull-eye inclusions and granular vesicles in human oocytes with evaluation of embryological, clinical and newborn outcomes after ICSI

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Study question: To determine if the presence of bull-eye inclusions and granular vacuoles in human oocytes affects the clinical outcomes after intracytoplasmic sperm injection (ICSI).

Summary answer: Transfer of embryos derived from dimorphic oocytes were associated with a significant decrease in clinical pregnancy (CP), live birth delivery (LBDR) and newborn (NB) rates.

What is known already: Several oocyte dimorphisms were related to poor ICSI outcomes: indented zona pellucida was associated with lower fertilization (FR), embryo cleavage (ECR) and blastocyst (BL) rates; large refractile bodies were associated with lower FR and BL rates; marked central dark granulation were associated with lower implantation (IR) and ongoing pregnancy (OP) rates and increase in embryo aneuploidy; single or multiple vacuoles were associated with lower FR; large aggregates of smooth endoplasmic reticulum tubules (aSERT) were associated with lower FR, ECR, BL, CP, OP, IR and LBDR rates, and NB deficiencies. The bull-eye inclusions and granular vacuoles have not been previously described.

Study design, size, duration: In this retrospective study we analysed all consecutive ICSI cycles (4099 cycles) performed during the period 2005–2013. Three groups were established: controls were those ICSI cycles whose oocytes showed no dimorphisms (3877 cycles, 3352 embryo transfers and 1218 NB); cycles with the presence of the bull-eye dimorphism (11 cycles, 0.27%, 10 embryo transfers and 5 NB); and cycles with the presence of the granular vacuole dimorphism (211 cycles, 5.15%, 203 embryo transfers and 75 NB).

Participants/materials, setting, methods: At a private ART clinic, patients were fully evaluated including karyotyping. Ovarian stimulation was mainly performed with a GnRH antagonist and rFSH, and HCG for oocyte trigger. Gamete and embryo handling followed previous descriptions. For luteal support we used progesterone, and estradiol and progesterone in cases where an agonist was used for oocyte trigger. Results were analysed by indicated statistics. Donated dimorphic oocytes were processed for transmission electron microscopy and in-situ fluorescence hybridization.

Main results and the role of chance: The ultrastructure of the bull-eye inclusion revealed a round prominent structure delimited by lipid droplets and made of small vesicles. As the ooplasm was devoid of large smooth endoplasmic reticulum vesicles and aSERT, it possibly to corresponds to an abnormal trapping

of SER. There were no significant differences to controls regarding demographic, stimulation, embryological, clinical and NB outcomes. There was 1 cycle with mixed embryo transfer (ET) with 1 NB and 1 cycle with pure ET (no NB).

The ultrastructure of the granular vacuole revealed a vesicle with a double membrane containing dense vesicles and lipid droplets. The presence of a double membrane raised the suspicion of micronucleus and in-situ fluorescence hybridization was performed, which revealed the presence of chromosomes. There were 141 cycles with ET of normal embryos (61 NB), 52 cycles with mixed ET (12 NB) and 10 cycles with pure ET (2 NB). There were no significant differences to controls regarding demographic, stimulation, embryological, clinical and NB outcomes. However, with intragroup analysis, mixed ET revealed lower CP (42.9% vs 7.9%), LBDR (32.5% vs 5.4%) and NB (32.5% vs 5.9%). Similarly, pure ET revealed lower CP (42.9% vs 2.0%), LBDR (32.5% vs 1.0%) and NB (32.5% vs 1.0%).

Limitations, reasons for caution: The limitation refers to the low number of cases with these dimorphisms. However, this is because they represent a rare event, with 0.3% of cycles exhibiting oocytes with bull-eye inclusions and 5.2% of cycles with granular vesicles, which occurred occurring during 10 years where there were 4099 consecutive ICSI cycles.

Wider implications of the findings: The presence of micronuclei would have a negative impact on embryo cleavage and implantation due to embryo aneuploidies. Absence of NB abnormalities in these cycles whose oocytes display granular vesicles might be linked to micronuclei chromosome degradation. Nevertheless, couples should be advised on the low probability of reaching a pregnancy.

Trial registration number: Not applicable.

P-153 DNA methylome analysis in mouse germ cells and early embryos

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Study question: Single resolution DNA methylome of mouse zygotes, blastocysts, and the primitive streak stage embryonic tissues has not been analyzed.

Summary answer: We performed DNA methylome analysis of mouse mature germ cells, zygotes, blastocysts, and post-implantation embryonic tissues.

What is known already: Recent studies proposed that demethylation occurs during the embryogenesis and gametogenesis via two different mechanisms, which may be passive or active mechanism. Both the events are thought to be essential for proper development; however, few details are available pertaining of demethylation.

Study design, size, duration: To understand epigenetic reprogramming that occurs after fertilization, we examined the genome-wide methylation profiles in mouse zygotes, blastocysts, and the primitive-streak-stage embryonic tissues by using Illumina sequencing libraries (n=2–5). A new whole-genome shotgun bisulfite sequeicing (WGSBS) library, termed the PBAT method, which accurately quantifies whole-genome methylation levels at a single-base resolution. The deep-sequencing data (average depth: 4.5~15.7) of each sample were obtained and analyzed.

Participants/materials, setting, methods: All gametes, zygotes and embryos were collected from C57BL/6N mice. Zygotes and normal blastocysts were produced by IVF. Successful fertilization and pronuclear stages were checked by Hoechst DNA staining. The polar bodies were removed by pipetting, and all samples were subjected to PBAT library construction and sequencing by Illumina HiSeq 2500. The sequenced reads were mapped to GRCm38/mm10 reference using Bismark.

Main results and the role of chance: Whole-genome DNA methylome maps of mouse germ cells and early embryos were generated by using amplification-free whole-genome bisulfite sequencing method (PBAT). Non-CpG methylation was observed in oocytes, zygotes, and epiblasts; however, the methyl cytosine (mC) distribution in epiblasts was different from that in oocytes, and was completely absent in the blastocyst stage (the non-CpG demethylation may take place via a passive process). Both parental genomes undergo global CpG demethylation during preimplantation development; however, marked demethylation of the paternal genomes was observed compared to the modest demethylation of the maternal genomes, and demethylation of PGC genomes is more intensive. Most of the known imprint control regions show the moderate mCpG levels in zygotes, blastocysts, and post-implantation embryos (via a highly orchestrated process that involves demethylation and *de novo* methylation during embryogenesis) due to the maintenance of their parent-of-origin methylation, and such regions (among the gdDMRs) are very rare.

Limitations, reasons for caution: Shown only one species, mice.

Wider implications of the findings: Our data could serve as a platform for future studies to elucidate the role of epigenetic modifications in the development and function of germline and stem cells.

Trial registration number: The trial number is 2 to 5.

P-154 Reduced early pregnancy loss of day 4 blastocysts transferred in artificial FET on P+5

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Study question: To investigate the reproductive outcomes of blastocysts that developed on day 4 of *in vitro* culture transferred on P+5 or P+6 of artificial FET cycles.

Summary answer: Transferring day 4 blastocysts on P+5 of FET results in excellent pregnancy and clinical pregnancy rates, and lower early pregnancy loss rates.

What is known already: Conventional embryo developmental milestones are important indicators of embryo competence, with fast or slow developing embryos having increased aneuploidy and consequently reduced implantation, however, fine-tuning of culture media and incubation technologies maybe changing the timing of some of these embryo developmental milestones. These changes may have important consequences for embryo-endometrium synchronization and optimal implantation.

Study design, size, duration: In this pilot study to a prospective randomized control trial, we retrospectively investigated cycles where blastocysts were vitrified on day 4 of embryo culture, between May 2015 and December 2015. The cycles were divided into two cohorts according to the day of embryo transfer, P+5 (d4-d4 group, $N = 53$) or P+6 (d4-d5 group, $N = 23$).

Participants/materials, setting, methods: SAGE 1-Step™ media (Origio, Denmark) was used for embryo culture and incubation conditions set at 6% CO_2, 5% O_2 and 37.0°C (K-Systems, Denmark). All inseminations were performed using ICSI. On day 4, embryos were checked at 92 hours and 100 hours. Vitrification and warming of blastocysts were performed using ultra-rapid technologies (Cryotop, Kitazato BioPharma Co. Ltd, Japan). In the artificial FET cycles endometrium-embryo synchronization was performed using progesterone supplementation (Crinone, Merck Serono, Turkey).

Main results and the role of chance: Since May 2015, an increasing number of patients have had blastocysts vitrified after 100 hours on day 4 of embryo culture. The following parameters were comparable between the d4-d5 group and the d4-d4 group, the mean patient age (31.5 ± 6.4 vs 31.5 ± 4.8 years), antral follicle count (16.9 ± 13.5 vs 17.8 ± 9.3), and number of blastocysts transferred (1.26 ± 0.45 vs 1.34 ± 0.48). The pregnancy and clinical pregnancy rates of the two groups were non-significantly different, 82.6% versus 84.9% ($p = 0.912$) and 69.6% versus 81.1% ($p = 0.835$), respectively. However, the early pregnancy loss rate was significantly higher ($p < 0.001$) in the d4-d5 group, 15.8% versus 4.4%, respectively.

Limitations, reasons for caution: A retrospective observational analysis.

Wider implications of the findings: In the subsequent RCT the reproductive outcomes of day 4 blastocysts may help to establish whether implantations are negatively affected by *in vitro* culture or whether the transfer of blastocysts on P+5 results in better embryo-endometrium synchronization – allowing implantation to occur within the optimal time of endometrial receptivity.

Trial registration number: N/A.

P-155 Standard blastocyst morphology is not a golden predictor of euploid embryos by correlation with preimplantation trophectoderm biopsies in women older than 35 during ICSI cycles

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Study question: Is conventional blastocyst morphological evaluation golden predictor of euploid embryos for women older than 35 in ICSI cycle by correlation with preimplantation trophectoderm (TE) biopsies?
Summary answer: There was no statistic correlation between the blastocysts morphology and euploid embryos by preimplantation trophectoderm biopsies in women older the 35.
What is known already: TE biopsy and comprehensive chromosome screening (CCS) is considered to be a promising approach to select euploid embryos for transfer. To kwon the role of morphology in blastocyst stage preimplantation genetic screening (PGS) cycles might be helpful in further IVF/ICSI cycles.
Study design, size, duration: This is a observational study performed between January 2014 and December 2014 in the reproductive center of Taiwan IVF group center, Taiwan. The study includes the data analysis of 581 blastocysts with conclusive CCS results obtained from 97 patients of 114 ICSI cycles.
Participants/materials, setting, methods: Preimplantation genetic screening was offered to infertile patients of advanced maternal age (>35 years) and/or with a history of unsuccessful IVF treatments or previous spontaneous abortion. Blastocyst morphology was assessed and categorized in three groups (excellent, average and poor). Linear regression models were used to test the relationship between blastocyst morphology and developmental rate CCS data and FET cycle outcomes of euploid blastocysts.
Main results and the role of chance: A total of 97 patients with 114 PGS cycles were included. There were 581 embryos analyzed. The overall euploid rate was 42.34% ($n = 246$). The implantation rate was 62.27%). In the excellent embryo group ($n = 314$), the euploid rate was 52.55% ($n = 165$); in the average embryo group ($n = 232$), the euploid rate was 31.47% ($n = 73$). In the poor embryo group ($n = 35$), the euploid rate was 22.86% ($n = 8$). There was no statistic difference between these three groups ($P > 0.05$). Also, there was no correlation between embryo morphology and embryonic euploid rate by CCS data ($P > 0.05$). In the present study, we found that the standard blastocyst morphology was not a golden predictor of euploid embryos in women older than 35 years old during ICSI cycles
Limitations, reasons for caution: The study is limited by its retrospective study. A larger sample size or a prospective randomized design could be used in future studies to corroborate the current findings.
Wider implications of the findings: This study provides knowledge that the standard blastocyst morphology evaluation are not good enough indicators to selection among euploid embryos in women older than 35 years old. Accordingly, all poor morphology and slower growing expanded blastocysts should be biopsied due to the similar chance of euploid embryos.
Trial registration number: None.

P-156 Effect of female age on early embryonic developmental speed before 1st cleavage

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Study question: Does female age have an impact on early embryonic development prior to 1st cleavage?
Summary answer: Female age was found to affect the tPNf and time interval of PNa-PNf. tPNf was faster and PNa-PNf was shorter with increasing female age.
What is known already: There are many reports about early development events predicting blastocyst formation after 1st cleavage, but knowledge of the most relevant biomarkers predicting blastocyst formation before 1st cleavage is still limited. Moreover, there is no information on the impact of female age on early embryonic developmental patterns prior to 1st cleavage.
Study design, size, duration: This prospective study examined 288 embryos resulting from normal fertilization in 45 patients undergoing ICSI over a 5 month period. Embryonic events were analysed in three age categories, less than 30 years old (Group A); 30–34 years old Group B), 35–39 years old (Group C).
Participants/materials, setting, methods: All embryos were cultured in the EmbryoScope® time lapse incubator, in droplets of 30µl of Continuous Single Culture Medium (Irvine Scientic, USA) until day 7. Time of 2nd polar body extrusion (tPB2), appearance (tPNa) and fade (tPNf) of pronuclei were analyzed. For defining tPNa, the time of maternal pronuclei appearance was used. The following intervals were calculated: PB2-PNa, PNa-PNf. Tukey test was used for comparison of mean timings. *P*-values < 0.05 were considered significant.
Main results and the role of chance: Regarding the average times of tPB2, tPNa and PB2-PNa, there was no significant difference between three groups. However for the average times of tPNf and PNa-PNf, there was a significant difference between Group A and C (24.4 h vs 22.8 h) (19.0 h vs 17.3 h) ($P < 0.05$). Moreover, analysing only those embryos which went on to form blastocysts, significant differences were observed between all 3 age groups for PNf and PNa-PNf (23.7 h vs 22.9 h vs 21.9 h)(18.4 h vs 17.5 h vs 16.6 h) ($P < 0.05$). Furthermore, significant differences in tPNf and the PNa-PNf timings were found between embryos which formed blastocysts and those which did not ($P < 0.05$). Within these 2 groupings, it was also observed that there was a significant difference in the tPNf and PNa-PNf between Group B and C ($P < 0.05$).
Limitations, reasons for caution: This study was limited to the analysis of embryos from Asada Ladies Clinic patients. Developmental speed of embryos can also be impacted by the culture environment, therefore these findings apply only to this patient group.
Wider implications of the findings: Patient age seems to be an important variable to consider when scoring embryo development events through time-lapse technologies.
Trial registration number: Not applicable.

P-157 Cryopreservation of oocytes for freeze-all policy in women at risk of ovarian hyperstimulation syndrome (OHSS): preliminary results.

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Study question: Can "oocyte freeze all" be proposed with satisfactory results for freeze-all strategy in cases at risk of OHSS?
Summary answer: Oocyte vitrification is rarely used in IVF programs although it appears from biological to ethical points of view an alternative to exclusive "embryo-freeze-all" policy.
What is known already: OHSS is the second complication of hormonal *in vitro* fertilization treatment. OHSS may happen during ovarian stimulation treatment or after embryo implantation. Late OHSS may be prevented avoiding pregnancy by freezing all embryos, also known as freeze-all policy. So far, this strategy proved its superiority to fresh embryo transfer in terms of clinical and obstetrical outcomes. For a majority of patients and professionals, oocyte cryopreservation appears to be ethically more acceptable than embryo freezing. However, "oocyte freeze all" remains still rarely used. Futhermore the efficiency of freeze-all strategy involving only oocytes has not yet been assessed
Study design, size, duration: Some expected complications of HOSS did permit embryo transfer. Since 2012, in cases at risk of OHSS, ovulation was triggered using either hCG or GnRH agonist and pregnancy was avoided by cryopreserving the entire cohort of mature oocytes. Oocyte warming cycles were proposed when clinical context allowed a pregnancy to occur. Embryos obtained after fertilization of warmed oocytes were replaced in context of spontaneous or using hormonal replacement therapy allowing better endometrial receptivity.
Participants/materials, setting, methods: 56 women (Mean age: 32.4 years ± 5.1) at risk of OHSS (Mean oestreadiol level: 3171.7 pg/ml ± 1358.0, mean number of mature oocytes vitrified: 19.0 ± 7.4) had the entire mature oocytes cohort cryopreserved using Kitazato vitrification kit. Among the women included in this study, 4% were at severe risk of HSO and 96% of moderate HSO. 50 of these women were proposed at least one oocyte warming cycle using Kitazato warming kit, corresponding to 100 warming cycles and 93 embryo transfers.
Main results and the role of chance: No hospitalisation or complication of OHSS was reported in the included patients. A total of 1063 oocytes were vitrified and to date 532 were warmed. 87(±18.5)% of warmed oocytes survived and underwent intracytoplasmic sperm injection (ICSI). A mean of 70(±20.7)% of injected oocytes showed 2 pronuclei 16–18 hours after ICSI. In 53.4% of the case embryo transfer was performed at embryo culture Day 5. Mean

number of embryo transferred was 1.6 resulting in early pregnancy rate per transfer (βHCG blood measurement>100UI/l) of 35.5% and clinical pregnancy rate (foetal heartbeat detected) of 32.3%. 2 miscarriages were reported before 12 weeks of gestational age. To date 11 deliveries gave birth to 13 healthy children and 17 pregnancies are still ongoing. It is important to note that 531 oocytes are still cryopreserved and available for warming cycle which provides supplementary pregnancy chances.
Limitations, reasons for caution: A larger cohort is needed to draw any definitive conclusion. Moreover, the present data obtained with oocyte vitrification need to be compared with the strategy involving only embryos.
Wider implications of the findings: These encouraging results may bring a tremendous change in freeze all policy giving an alternative to exclusive embryo freeze-all. Furthermore, the major advantage of oocyte vitrification is to give couples the opportunity to have more than only one ICSI cycles allowing fresh embryo transfer without ovarian stimulation and its complications.
Trial registration number: None.

P-158 Anti-oxidative effect of 6,9,12-hexadecatrienoic acid on *in vitro* development of pre-implantation embryos

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Study question: Does supplementation of culture media with 6,9,12-hexadecatrienoic acid improve *in vitro* development of pre-implantation embryos under oxidative stress?
Summary answer: 6,9,12-hexadecatrienoic acid supplementation may rescue embryos under oxidative stress by inhibiting reactive oxygen species (ROS) production and increasing mitochondrial membrane potential (MMP).
What is known already: Oxidative stress, especially ROS, has detrimental effects on embryo development. Although supplementing culture media with antioxidants such as melatonin, vitamin E and pyruvate has been reported to improve the quality and developmental rate of animal embryos, these substances are not widely used in clinical settings. Recently, eicosapentaenoic acid, a type of polyunsaturated fatty acid (PUFA), was shown to prevent tetrachlorodibenzodioxin-induced oxidative stress in HepG2 cells. In a preliminary study, 6,9,12-hexadecatrienoic acid, a type of PUFA, also improved *in vitro* development of mouse embryos under oxidative stress.
Study design, size, duration: We studied 167 pre-implantation mouse zygotes and 20 donated pre-implantation 4-cell human embryos *in vitro*. Mouse zygotes were pre-incubated with 6,9,12-hexadecatrienoic acid in dimethyl sulfoxide (treated group, $n = 57$) or dimethyl sulfoxide alone (negative controls, $n = 54$) before H_2O_2-induced oxidative stress, or not exposed to 6,9,12-hexadecatrienoic or oxidative stress (non-treated controls, $n = 55$). Human embryos were treated with 6,9,12-hexadecatrienoic acid (treated group, $n = 10$) or not exposed to 6,9,12-hexadecatrienoic acid (non-treated controls, $n = 10$).
Participants/materials, setting, methods: 6,9,12-hexadecatrienoic acid was purified from pacific krill (*Euphausia pacifica*). We used morphological and cytochemical analysis to assess embryo development and ROS production, respectively. We also analyzed MMP at the morula stage, total cell number at the blastocyst stage, and the ratio of inner cell mass (ICM) to trophoectoderm (TE) at the blastocyst stage. Fluorescence of ROS production and MMP were calculated using Image J software. Values are expressed as mean ± standard error of mean.
Main results and the role of chance: Under H_2O_2-induced oxidative stress, the rate of blastocyst formation in mouse zygotes treated with 6,9,12-hexadecatrienoic acid was higher than in negative controls (58.8% v 25.0%; non-treated controls, 70.6%). For ROS, relative fluorescence intensity per embryo 24 hours after H_2O_2 exposure was lower in the treated group than in negative controls (9.9 ± 1.0 v 11.6 ± 0.5; non-treated controls, 7.9 ± 0.5). For MMP, relative fluorescence intensity per embryo (measured using tetramethylrhodamine methyl ester) was higher in the treated group than in the negative controls at the morula stage (1.083 ± 0.08 v 1.008 ± 0.07; non-treated controls, 1.000 ± 0.08). The total cell number and the ICM:TE ratio at the blastocyst stage was not significantly different between groups. The rate of blastocyst formation did not differ between the treated and non-treated human embryos (80.0% v 70.0%), but the hatching rate for the human embryos was much higher in the treated group (70.0% v 20.0%).
Limitations, reasons for caution: Further investigations with larger samples are needed to confirm our results. The molecular mechanism that underlies the antioxidant effect of 6,9,12-hexadecatrienoic acid is unclear.
Wider implications of the findings: 6,9,12-hexadecatrienoic acid could be used as a safe and effective functional food ingredient, and might become a useful supplement for reducing oxidative stress during *in vitro* culture of embryos and while obtaining viable embryos for assisted reproductive technology.
Trial registration number: NA.

P-159 Embryonic estrogen receptors: do they have a role in the implantation and post-implantation development?

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Study question: Do estrogenic hormones in the blastocysts have a physiological role in the implantation and post-implantation development?
Summary answer: The implantation and post-implantation development of mouse blastocysts *in vitro* were adversely affected by blocking estrogen receptors (ER) using ER blockers.
What is known already: The effects of estrogen depend on the existence of their specific receptors. ER mRNA was found in oocytes and fertilized eggs. The message level began to decline at the two-cell stage and reached its lowest level at the five- to eight-cell stage. ER mRNA reappeared at the blastocyst stage. The embryonic expression of ER genes in the blastocyst suggests a possible functional requirement for ER at this stage of development. These results provide a basis for determining the direct role of estrogen in pre-implantation embryos.
Study design, size, duration: The physiological function of the ER was evaluated by using the ER blockers. Mice blastocysts were cultured for 8 days to evaluate the morphological development. 1000 blastocysts were collected from the ICR female mice for the *in vitro* culture. 100 blastocysts were collected for the immunocytochemical assay to detect the ER in the pre-implantation embryos.
Participants/materials, setting, methods: This was an *in vitro* experimental study involving the use of mouse blastocysts and the model of in-vitro 8-day culture. Under the tamoxifen (ER-α blocker) or ICI182,780 (ER-α/-β blocker) r PHTPP (ER-β blocker) treatment, implantation and post-implantation development were evaluated daily. The ER proteins in the pre-implantation and post-implantation embryos were detected through the immunocytochemical assay.
Main results and the role of chance: The ER-α and β were noted in the blastocyst and early post-implantation embryos (implanted blastocysts and early egg cylinder stage embryos). However, only ER-β was noted in the late egg cylinder stage embryos. Differential expression of ER in the post-implantation embryos was shown in the study. At the concentration of 10^{-7}M or more, tamoxifen impaired the development of the late egg cylinder stage and early somite stage. Furthermore, at the concentration of 10^{-5}M, the adverse effects occurred earlier at the stage of early egg cylinder embryos. Similar effects of ICI182,780 were found at the concentration of 10^{-8}M to 10^{-6}M. Furthermore, the development from implanted blastocysts to early somite embryo was completely affected adversely at the concentration of 10^{-5}M of ICI182,780. PHTPP did not impair the development of implantation and early egg stage embryos. However PHTPP at the concentration of 10^{-7}M to 10^{-5}M impair the development of late egg cylinder stage embryo. In conclusion, the dose-dependent adverse effects of ER blocker on the implantation and post-implantation development were demonstrated clearly. Reviewing the literatures, this is the first piece of evidence that physiological role of ER in the blastocysts and post-implantation were demonstrated in the study.
Limitations, reasons for caution: Considering the ethical and technical limitations inherent to the use of human embryos for implantation studies, the mouse

model was used as an approach for exploring the potential role of embryonic ER on the implantation and post-implantation embryo development. Nevertheless, the extrapolation of these results to humans requires further investigation.
Wider implications of the findings: This study presents clear evidence on the potential role of embryonic ER on the implantation and post-implantation embryo development. These new findings could contribute to the physiological knowledge of human embryo culture for the clinical applications in in-vitro – fertilization protocol.
Trial registration number: The study has been supported by grants from the National Science Foundation of Taiwan (NSC-101-2314-B-182A-110, NSC-102-2314-B-182A-085 and NSC-103-2314-B-182A-108). There is no competing interest.

P-160 Time-lapse imaging provides further evidence that planar arrangement of blastomeres is abnormal

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Study question: Are developmental kinetics of planar human embryos different from tetrahedral ones?
Summary answer: Planar embryos are associated with both a significant increase in irregular cleavage as well as a remarkable delay in preimplantation development.
What is known already: Normal day-2 embryos show a tetrahedral arrangement of four cells with three blastomeres lying side by side. Cleavage anomalies include embryos that are characterized by a particular planar constellation of four blastomeres with presumed incomplete cleavage. The fact that such embryos show a reduced implantation capacity led an experienced time-lapse user group to recommend the annotation of a planar arrangement of cells on observation (tPA). However, to date no information on morphokinetics of planar embryos is available.
Study design, size, duration: Within a 5-month study period (August to December 2015) all consecutive patients were prospectively screened for day-2 planar embryos. Planar embryos were annotated according to generally acknowledged and novel time-lapse variables. Morphokinetic parameters of the sibling embryos showing normal cleavage pattern served as a control.
Participants/materials, setting, methods: A total of 210 patients frequented an university-affiliated clinic in the study period. The rate of affected patients (exclusively ICSI cases) and embryos was 12% and 4%, respectively. All embryos were cultured in a novel time-lapse incubator in a tri-gas milieu until day 5. A 5-minute picture interval was set to optimize annotation.
Main results and the role of chance: Significantly more ($P > 0.001$) planar embryos showed morphological (e.g., bi- and multi-nucleation) and morphokinetic (trichotomous mitosis, cell fusion, embryo rolling) anomalies as compared to tetrahedral embryos. Time-lapse imaging allowed to deselect 45% of the planar and 13% of the normal embryos (this information would have been missed using static observation). Morphokinetics were unaffected up to 4-cell stage, thereafter planar embryos cleaved significantly slower (3–4 h) as indicated by t5 to t8.
Limitations, reasons for caution: This is the first study using a time-lapse incubator with individual incubation chambers. It is not clear to what extent morphokinetic variables may differ from those assessed with alternative time-lapse-incubators. It should be kept in mind that, theoretically, patients generating one or more planar embryos could have an intrinsic problem.
Wider implications of the findings: Time-lapse imaging of human day 2 planar embryos ultimately confirms what has been hypothesized based on static observations of the same. Increased presence of multinucleated blastomeres and irregular cleavage behavior strongly indicate that planar embryos are abnormal and should be deselected.
Trial registration number: None.

P-161 No differences in development/quality of embryos cultured in sequential and single-step medium regarding of female age and method of *in vitro* fertilization: prospective study

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Study question: What is the difference in embryo development and quality after prolonged *in vitro* culturing to the blastocyst stage in sequential media and single-step medium?
Summary answer: No differences in the development of embryos to the blastocyst stage and their quality were found after culturing in sequential media and single-step medium.
What is known already: Sequential media protocols are sufficient but not necessary to support the complete *in vitro* development of human preimplantation embryos. It has already been published that human embryos can be successfully cultured from day 0 onward in single-step medium without renew it on Day 3. In spite of this, there is still some scepticism about the single-step medium maybe to be sufficient for embryo development to the blastocyst stage.
Study design, size, duration: In this prospective, sibling zygote/embryo study, we compared the development and quality of embryos, cultured in sequential media and single-step medium, regarding of female age and method of *in vitro* fertilization (IVF or ICSI). A total of 581 normally fertilized (2PN) zygotes from 96 IVF/ICSI cycles in 96 couples were randomly allocated to two culture systems: sequential media (285 zygotes) and single-step medium (296 zygotes) to compare the embryo development and quality.
Participants/materials, setting, methods: In each couple, a half of embryos was cultured in Sydney IVF COOK sequential media (Cleavage medium (Day 1–3) and Blastocyst medium (Day 3–5/6) and another half of embryos in SAGE 1 single-step medium (Origio) from Day 1 to Day 5/6 to compare the blastocyst rate and the quality of derived blastocysts according to Gardner (2000). The collected data were analyzed using Chi-Square test. Statistical significance was set up at $P < 0.05$.
Main results and the role of chance: The derived blastocysts were assigned to one of three quality groups in terms of development and morphology of embryoblast and trophectoderm: 1) good-quality (≥3AA), 2) fair-quality (≥3AB and BA), or 3) poor-quality (1–6 BB, CC) blastocyst. The blastocyst rate and the proportion of good-quality blastocysts, developed in sequential media, were not significantly different from those, developed in single-step medium (43.6 vs. 44.8% and 48.3 vs. 48.1%, respectively). There were no statistically significant differences in the proportion of fair-quality blastocysts (13.9 vs. 17.5%) and poor-quality blastocysts (37.7 vs. 34.3%) between the two culture systems. In addition, there was approximately the same proportion of morulae (17.5 vs. 17.1%) and arrested embryos (38.9 vs. 38%) in both culture systems. And finally, there was no statistically significant differences in blastocyst quality between the two culture systems regarding of female age (≤36/>36 years) and method of *in vitro* fertilization (IVF, ICSI).
Limitations, reasons for caution: The differences in groups failed to reach statistical significance. We have to put attention on implanted embryos and the outcome of pregnancy in the future.
Wider implications of the findings: The results of this study indicate that the sequential media are quite comparable to single-step medium in terms of embryo development and quality. Therefore the sequential media can be replaced by single-step medium to culture embryos to the blastocyst stage in daily clinical practice.
Trial registration number: 0.

P-162 Egg age modulates calcium signaling pattern, specific histone modification and embryonic development outcome in the mouse

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Study question: Egg age is a crucial factor affecting embryo quality, and thus closely associated with the outcome of embryo development. However, it is unclear how egg age affects the embryo development.
Summary answer: Egg age affects the pattern of calcium signaling at fertilization, epigenetic modifications and sperm chromatin decondensation, and thus may modulate the fetal and placental development.
What is known already: In mammals, ovulated eggs arrested at MII stage are normally fertilized soon after ovulation. If fertilization does not occur within the narrow optimal window, unfertilized eggs that remain in the oviduct would lead to aging. egg age is one of the key factor affecting the egg's quality and embryo's developmental potential after fertilization.

Study design, size, duration: Mature mouse eggs were collected from oviducts at 11–12 h, 14–15 h, 19–20 h after hCG injection. Then, eggs were fertilization by fertilized by intracytoplasmic sperm injection (ICSI). The relationship between egg age and embryonic development, the pattern of calcium signaling at fertilization, the timing of pronuclear formation, and epigenetic modifications were analysed.
Participants/materials, setting, methods: CD1 strain mice (6–8 weeks old) were used. Intracytoplasmic injection of spermatozoa was performed using a Leica Inverted Microscope equipped with a pair of manually operated pressure microinjector (IM-6 Narishige) and Piezo-system (PMM Controller, Model PMAS-CT150). The cytoplasmic Ca^{2+} of mouse eggs was measured using a Mira-Cal Ratio Vision digital fluorescence imaging system. H3K9 acetyl, H3K9 methyl, H4K16 acetyl, H3T11 phos were analysed by immunofluorescence staining.
Main results and the role of chance: 1) egg age significantly decreased blastocyst formation rate, blastocyst total cell number, the normal fetuses rate, and placental weight; 2) aged eggs had an abnormal pattern of calcium signaling at fertilization, significant increased eggs showing only 1–2 calcium spike in comparison with young eggs; 3) egg age affected the timing of pronuclear formation; 4) H4K16 acetylation in aged eggs decreased significantly quickly in sperm chromatin following fertilization; 5) aged eggs showed a significantly reduction in sperm chromatin decondensation by judging the area of sperm nucleus.
Limitations, reasons for caution: This study was carried out using a mouse model. Whether egg age affects the fertilization events in human and affect the development of human fetal and placental development need further study.
Wider implications of the findings: Egg age affects the development of mouse embryonic development partly via the variation of calcium signaling pattern and specific histone modification.
Trial registration number: Basic animal research.

P-163 Clinical outcomes and development of children born from vitrified oocytes for azoospermic patients

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Study question: The aim of this study was to assess the effect of oocyte vitrification on clinical outcomes and development of childlen compared to fresh oocytes.
Summary answer: There were no significant differences in clinical results or child development between fresh and vitrified oocytes.
What is known already: Vitrification has been reported to be a simple, cost effective, efficient method for cryopreservation of mammalian and human oocytes. Many studies have shown more positive results with oocyte vitrification than with slow freezing procedures.
Study design, size, duration: Retrospective study. Subjects were 438 couples with 924 oocyte-retrieval cycles for azoospermic patients from Dec., 1996 to Sep., 2015. Children were born from vitrified oocytes (68 from 66 pregnancies) and fresh oocytes (230 from 196 pregnancies). Singleton and multiple pregnancies from the husband's sperm and donated sperm were included.
Participants/materials, setting, methods: Oocyte vitrification by the Cryotop method has been performed in our clinic since 2003. We assessed the cumulative pregnancy rate, neonatal conditions at birth, and development of movement and language between the two groups from ages 1 to 6 years old.
Main results and the role of chance: In the vitrified group, the survival rate of oocytes after warming was 87.2% (909/1043). Mean women's ages were 33.6 ± 4.8 and 33.1 ± 6.6 at oocyte retrieval and mean men's ages were 35.7 ± 4.9 and 34.9 ± 5.8 in the vitrified and fresh groups, respectively. No differences were found between the two groups in pregnancy rate (35.2%:93/264 vs. 36.5%:364/996), gestational age at delivery (39.4 weeks vs. 39.2 weeks), birth weight (3.070 g vs. 3.067 g), birth height (39.4 cm vs. 39.2 cm). In the vitrified group, there were no cases of congenital abnormality. In the fresh group, there were two cases of congenital abnormality: one of polymelia and one of atrial septal defect. There were no differences in movement and language development between the two groups.
Limitations, reasons for caution: None.
Wider implications of the findings: Regarding oocyte vitrification, many studies have shown more positive results than with slow freezing procedures. In this study, there are no significant differences between fresh and vitrified oocytes for azoospermic patients. The use of vitrified unfertilized oocytes should be recommended for wide medical treatment.
Trial registration number: None.

P-164 The utilization of different gonadotrophin preparations induces significant differences in the generation of high quality embyos as measured by time lapse technology

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Study question: To evaluate if different protocols of stimulation influence embryo morphology dynamics and implantation rates in patients undergoing IVF/ICSI for the treatment of infertility.
Summary answer: Different gonadotrophin protocol induces significant differences in the generation of high quality embryos. The higher proportion of high quality embryos is observed with r-FSH + r-LH.
What is known already: The impact of protocols of stimulation on the quality of the embryos generated is a topic of continuous discussion. Most studies comparing gonadotrophins, assess embryo quality and implantation potential utilizing single point morphological evaluations which are subjective and may change between different IVF settings. To be able to identify the best protocol of stimulation for each patient and maximize the production of high quality embryos, it is of outmost importance to be able to assess quality with an objective technology based on time-lapse.
Study design, size, duration: Retrospective cohort study. We examined 650 *in vitro* fertilization-intracytoplasmic sperm injection (IVF/ICSI) cycles in patients with controlled ovarian stimulation utilizing different gonadotrophin protocols by combining two recombinant gonadotrophins (r-FSH+r-LH) (n = 91), only r-FSH (n = 331) or with hMG (n = 228). A total of 3128 embryos were cultured in single step medium and recorded from zygote to blastocyst stage analyzed in Embryoscope™ time-lapse technology (Vitrolife, Sweeden). The study period were between October 2011 and November 2015.
Participants/materials, setting, methods: Ovarian stimulation of patients analyzed were performed with three different gonadotrophin protocols as described before. After ICSI, oocytes and embryos. High quality embryos were defined as those with optimal morphokinetic development and high implantation potential, as described by Vermilyea et al. (2014). The variables analyzed by multiple logistic regression included the duration and the synchrony of the second embryo cell cycle together with the number of oocytes retrieved, maternal age and body mass index (BMI).
Main results and the role of chance: Descriptive characteristics of the patients in the stimulation protocols groups included in the analysis revealed no differences maternal age and BMI. Also Estradiol and Progesterone levels on the day of hCG administration were comparable together with similar days of stimulation. On the contrary the number of oocytes retrieved were 12.9 (12.4–13.5, r-FSH), 9.1 (CI95% 8.3–9.9, hMG), 9.9 (8.8–8.11.0, r-FSH+r-LH) and Metaphase II oocytes 7.7 (CI95%7.1–8.3), 9.8 (CI95%9.3–10.3) and 7.9 (CI95%7.0–8.9) respectively for r-FSH, hMG and r-FSH+r-LH. Different gonadotrophin protocols of stimulation induces significant differences in the proportion of high quality embryos observed (r-FSH = 7.35%, hMG = 7.86%, r-FSH+r-LH = 12.18%; p = 0.016). Multiple logistic regression analysis revealed significant effect produced by gonadotropin protocol on the production of high quality embryos (r-FSH+r-LH vs hMG, odds ratio (OR) = 1.876 (CI95%1.17–3.02) p = 0.010). The multivariable analysis also revealed that the number of oocytes recovered, maternal age and BMI did not prove to be an influential factor on embryo quality. There were also relevant differences on implantation rate related with stimulation protocols (r-FSH 33.3%, hMG 27.1%, r-FSH+r-LH 35.7%), but these differences were not significant, probably due to the still reduced sample size.
Limitations, reasons for caution: Although the multiple regression analysis is controlling all the potential confusing factors that may bias the current data the retrospective nature of the present study should take our conclusions with precaution. Future prospective randomized studies would be ideal to confirm significant differences in embryo quality and implantation rate.
Wider implications of the findings: The outcome of our study suggest that different gonadotrophin protocols induces significant differences in the

generation of high quality embryos. Customized stimulation protocols adapted to the clinical features of our patients may lead us with the generation of higher proportion of good quality embryos enhancing reproductive outcome.
Trial registration number: None.

P-165 Computer-automated time-lapse analysis can be use with two distinct levels of oxygen concentration

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Study question: The aim of this study was to analyse if computer-automated prediction scores for embryo development potential also correlate to patient pregnancy using two different levels of oxygen concentration.
Summary answer: Eeva High and Medium embryos have a significantly higher likelihood of get a pregnancy than Eeva Low embryos irrespective of the oxygen concentration used.
What is known already: The Eeva™ Test (Early Embryo Viability Assessment) is a prognostic test based on automated detection and analysis of time-lapse imaging information and it has been shown to benefit embryo selection specificity when used adjunctively with traditional morphology. The classification model was based on two cell division timing parameters, both of which have been shown to consistently correlate to embryo development. However, the methods above have not yet been validated using incubators with different oxygen concentration.
Study design, size, duration: This is a retrospective study including 349 embryos from 49 patients who were undergoing fresh *in vitro* fertilization (IVF) treatment. 26 patients used their own eggs (53.1%) embryos and 23 patients used donor eggs (46.9%). All of them consented to have their embryos imaged using the Eeva System from January 2015 to December 2015.
Participants/materials, setting, methods: Computer-automated assessment of 349 embryos was performed using the Eeva Test. 222 embryos (63.6%) were cultured with a 5% Oxygen concentration and 127 (36.4%) with a 20% oxygen concentration. The relationship between such computer-derived outputs (High, Medium, Low scores), and clinical pregnancy were examined at 5% and 20% oxygen tension
Main results and the role of chance: The EEVA result were: 63 (18.1%) No Result, 147 (42.1%) Low, 58 (16.6%) Medium and 81 (23.2%) High. There were no significant difference with the EEVA Score when we studied the eggs source (chi2 *p*:0.796) neither oxygen concentration of the incubator we used (chi2 *p*:0.202).

We transferred a total of 75 embryos, from which 21 (28%) were Low, 19 (25.3%) were Medium and 35 (46.7%) were High. When we applied an univariate analysis we found a positive linear correlation (*p*: 0.037) between scores and pregnancy, 54.3%, 52.6%, 23.8% pregnancy rates to High, Medium and Low Scores respectively. Moreover, if we limit the Eeva score in two categories, High-Medium and Low, the statistic significance in pregnancy rate will increase to 53.7% vs 23.8%.

In our case a binary logistic regression multivariate model helped us to discard the concomitant effect of confusion factors and we could check that eggs source and Eeva score were two independent forecasts factors each other and with the confusion factors introduced at model.

Considering this confusion factors, the donor eggs give us more probability to achieve a pregnancy with a OR 6.379 (*p*:0.003) and also the embryo transfer with a High or Medium Score with a OR 3.933 (*p*:0.035).
Limitations, reasons for caution: The different oxygen concentration is a choice that the laboratories have to do, and many studies demonstrate that this concentration varies the morphokinetic parameters of embryos. However, in our study we have not observed difference. Obviously, this study needs more cases to be consolidated.
Wider implications of the findings: Our results show that it's the same oxygen concentration and the Eeva results doesn't vary whith donor or patient eggs. According with our results, it's possible to unify High and Medium Eeva Score like some years, but with wide time limits, we have the risk to do a misclassification.
Trial registration number: No clinical trial.

P-166 Is day 5 transfer still necessary in the time-lapse era?

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Study question: Are there differences in morphokinetics between embryos that did not implant and those resulting in live births?
Summary answer: Embryos resulting in live births presented significant morphokinetic differences in the first three days of culture in comparison with not implanting embryos.
What is known already: Time-lapse imaging allows exact observation of morphokinetic characteristics of embryos during its culture. Several studies have developed algorithms to predict embryo blastulation, implantation or aneuploidy. Many authors reported better pregnancy and implantation rates, as well as less miscarriage. However, as far as we are concerned, no study has ever compared the morphokinetics of embryos in extended *in vitro* culture resulting in live births with those not implanted.
Study design, size, duration: This was a retrospective study of 272 embryos from 168 patients attending a private fertility clinic in Germany between April 2012 and June 2013. Embryos were divided into two groups – no implantation (IF, $n = 222$) or 100% implantation and live birth (LB, $n = 52$). For analysis, 23 morphokinetic parameters were selected and compared among the embryos.
Participants/materials, setting, methods: Only fresh stimulated ICSI cycles were included in the analysis. Embryos were cultured in the EmbryoScope after intracytoplasmic sperm injection (ICSI, time zero) in sequential media at low oxygen concentration. Embryo transfer (ET) was performed on day 2 to 6 depending on the number of available embryos. Selection of embryos for ET was based solely on morphology before ET. Kruskal-Wallis or Mann Whitney test were used and $p < 0.05$ was considered significant.
Main results and the role of chance: To discard a possible effect of age on morphokinetics, embryos were divided according to female age in three groups (≤ 34, 35–39 and ≥ 40 years) but no significant differences among the groups could be seen. On the other side, when analyzing the morphokinetics of IF and LB embryos, we could observe that seven parameters differed significantly between the groups. Results are presented as mean of hours post ICSI ± standard deviation. Firstly, syngamy in IF embryos occurred later than in LB embryos, 24.09 ± 3.49 versus 22.92 ± 2.81 ($p = 0.0196$), respectively. Secondly, time to reach the 2-cell stage (t2), a well established good prognosis marker before time lapse, also differed slightly between IF and LB embryos (26.96 ± 3.87 versus 26.27 ± 4.78, respectively, $p = 0.0313$). Additionally, embryos in the IF group seemed to be slower than in the LB group in their development regarding time to reach the 5-, 6- and 7-cell stage (t5, $p = 0.0193$; t6, $p = 0.0032$; t7, $p = 0.0002$). Thus, the duration of the third round of embryonic cleavage (cc3) was also longer in the IF than LB group ($p = 0.0015$). Furthermore, start of blastulation (tFB) occurred earlier in the LB group (100.4 ± 5.72) than in the IF group (103.4 ± 7.323, $p = 0.0015$).
Limitations, reasons for caution: This is a retrospective analysis in a small group of patients. Possible confounding factors, such as length of infertility, were not adjusted when doing the analysis. Furthermore, the use of algorithms for embryo selection still requires further studies before being applied in routine practice.
Wider implications of the findings: *In vitro* culture has been suggested to affect not only later embryo development but also perinatal outcomes. Developing an algorithm that allows successful embryo selection within three days of culture could minimize possible negative effects by reducing culture period and also reduce costs for patients.
Trial registration number: Not applied.

P-167 Low oxygen tension may increase implantation potential by enhanced expression of hypoxia and antioxidant genes in mouse blastocyst cultured *in vitro*

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Study question: How low O_2 tension affect the embryo viability, and gene expression profile cultured *in vitro*?

Summary answer: The lower O_2 tension improve embryo viability by increased antioxidant enzymes and LIFR to increase implantation ability, enhanced mitochondrial activity during implantation period.
What is known already: In human IVF, the oxidative stress appears to jeopardize *in vitro* embryo development. Many studies showed lower oxygen concentration had as a better result as well as addition of antioxidants to culture media. However the mechanisms of beneficial effects of reduced oxygen tension in embryogenesis remain unclear.
Study design, size, duration: A prospective RCT of experimental animal study in a university hospital.
Participants/materials, setting, methods: The 2-cell ICR mouse embryos were cultured to blastocyst stage under 3% O_2(n = 330) tension and 20% O_2(n = 317) tension. The expression of antioxidant genes (MnSOD and PRDX5) were analyzed by real-time RT PCR and validated by Immunofluorescence. The apoptosis, mitochondrial membrane potential (mtMP) and ROS level were assessed by TUNEL, stain with JC-1 and DCFDA, respectively.
Main results and the role of chance: The blastocyst formation and hatching rate increased significantly in 3% O_2 group when compared to 20% O_2 group (P < 0.5). The transcription levels of MnSOD and PRDX5 were also significantly increased seven to eight fold in 3% O_2 group, compared to 20% O_2 group (P < 0.05). Immunofluorescence staining showed the intensity of HIF-2α, MnSOD and LIFR was higher in 3% O_2 than 20% O_2 group, respectively. Apoptosis and ROS levels was significantly higher in 20% O_2, compared with 3% O_2 group (P < 0.05). The 3% O_2 blastocyst also showed a significantly higher mtMP compared with 20% O_2 group.
Limitations, reasons for caution: Our study was based in mouse model and further studies on implantation ability or birth rate are required to confirm our findings.
Wider implications of the findings: This study provided new evidence of a beneficial effect of 3% oxygen tension in embryonic competence and may improve further implantation; it may also assist in developing best culture condition for IVF.
Trial registration number: NA.

P-168 Effect of timing of oocyte denudation after oocyte retrieval on fertilization and pregnancy outcome following cleavage stage single embryo transfer

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Study question: Does denuding timing of cumulus-ooycte complexes after oocyte retrieval (OR) for ICSI influence fertilization rate and pregnancy outcomes following single embryo transfer?
Summary answer: Fertilization and clinical pregnancy rates were significantly declined as duration between OR and oocyte denuding became longer.
What is known already: It has been hypothesized that cumulus cells secrete paracrine factors which promote post-ovulatory oocyte aging. This event potentially concur with the entry of cumulus cells into apoptosis. Additionally, previous findings that the degree of apoptosis in cumulus cells in matured oocytes influence the fertilization rate suggest the possible correlation between timing of oocyte denudation after OR and pregnancy outcomes. Currently, in ART, little is known regarding the effect of duration between OPU and oocyte denudation on fertilization and pregnancy outcomes. Therefore further IVF laboratory based studies were required to clarify the hypothesis.
Study design, size, duration: A retrospective cohort study of 302 IVF cycles (566 oocytes) was conducted in single center from March 2015 to November 2015. The timing of denudation after OR was classified into three groups: less than 2 hours (Group A, n = 80), 2–3 hours (Group B, n = 165) and 3 hours and over (Group C, n = 57). Fertilization rate after ICSI and clinical pregnancy rate with gestational sac following single cleavage stage embryo transfer (SET) were compared among the groups.
Participants/materials, setting, methods: This cohort fulfilled the following criteria: woman's age at egg retrieval was 33–39 years, less than 3 times of repeated cycle and the less than 5 oocytes for ICSI. The oocytes were retrieved by minimum stimulation or natural cycles. Spindle position was checked prior to ICSI by a polarized light optical system. Outcome measures were fertilization rate (2PN), abnormal fertilization rate and clinical pregnancy rate following SET in 163 SET cycles.
Main results and the role of chance: There were significant differences in fertilization rate (2PN) between Group A and Group C (A: 91.2% 114/125, B: 87.3% 288/330 and C: 82.0% 91/111, p < 0.05). Abnormal fertilization rate was similar among the groups (A: 1.6% (3/125), B: 2.4% (8/330) and C: 4.5% (5/111)). However, abnormal fertilization rate was tended to increase as duration between OR and denuding became longer. Clinical pregnancy rates following subsequent SET were not significantly different between groups (A: 38.9% 23/59, B: 26.9% 21/78, C: 23.0% 6/26), yet, there was a tendency to improve the clinical pregnancy rate in earlier denuding group.
Limitations, reasons for caution: The main limitation of the present study is related to its retrospective nature and to a relatively small sample size.
Wider implications of the findings: Our finding that normal fertilization and clinical pregnancy rates were improved as duration between OPU and oocyte denuding became earlier suggests that timing of oocyte denuding could influence the fertilization and pregnancy outcomes. Therefore, cumulus-oocyte complexes are recommended to be denuded early after OR in the case of ICSI.
Trial registration number: None.

P-169 The assessment of metaphase-I (MI) oocytes retrieved from COH by time-lapse imaging

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Study question: The aim of this study was to evaluate the fertilization potential and morphokinetics of MI oocytes from controlled ovarian hyperstimulation cycles of patients.
Summary answer: To compare the metaphase-II (MII) oocytes, MI-derived embryos decreased the fertilization rate. Moreover, MI oocytes were significantly slower than MII oocytes of morphokinetics.
What is known already: During human assisted reproductive technology, about 20% of oocytes fail to reach the MII stage within 38 hours after administration of hCG. The use of immature oocytes could increase the number of embryos obtained to enhance the chance of pregnancy. However, there is a low fertilization rate when ICSI is performed with immature oocytes, with the potential for abnormal embryonic development due to defective cytoplasmic maturation. Until now, reports of morphokinetics and abnormal embryo events from hyperstimulated MI oocytes are mostly limited.
Study design, size, duration: This retrospective study on 87 patients performed between January 2014 and September 2015. Sibling embryos (n = 703, MII oocytes vs n = 231, MI oocytes) from the same patient were cultured in time-lapse incubator. This study was performed in 92 cycles that had undergone ICSI treatment.
Participants/materials, setting, methods: MII and MI oocytes were obtained from controlled ovarian hyperstimulation cycles of patients. MII oocytes were performed ICSI for 4–5 h after oocyte retrieval, while MI oocytes were cultured in maturation media and then were performed ICSI after checking the maturity. All embryos were cultured after ICSI assessed in a time-lapse incubator (EmbryoScope, Unisense Fertilitech, Denmark) and annotated for pattern time of cleavage.
Main results and the role of chance: The fertilization and good quality embryo rate of MI-derived embryos were significantly lower than that of MII oocytes (63.6% vs 76.5% and 44.9% vs. 62.0%, respectively; P < 0.001). There was no difference in the rates of multinucleation at the 2-cell stage (37.2% vs. 37.4%, P = 0.957). However, the uneven blastomere size at the 2-cell stage (39.5% vs. 24.0%, P < 0.001) and direct cleavage from 1 to 3-cell (32.7% vs. 16.9%, P < 0.001) showed statistically significantly higher rates in MI oocytes compared to MII oocytes. The mean time-points for MI oocytes-derived embryos and MII oocytes respectively were; tPB2 (4.4 vs 3.0, P < 0.001), tPNf (27.7 vs 24.4, P < 0.001), t2 (29.7 vs 26.9, P < 0.001), t3 (38.6 vs 36.6, P = 0.014), t4 (41.2 vs 39.0, P = 0.002), t5 (51.6 vs 49.4, P = 0.021), t6 (54.8 vs 52.4, P = 0.013), t7 (58.5 vs 54.9, P = 0.003), t8 (61.6 vs 57.9, P = 0.013), cc2 (8.2 vs 9.6, P = 0.570), s2 (2.9 vs 2.6. P = 0.579).

Limitations, reasons for caution: The study was a retrospective study. Data set size is small numbers. It should be extended to larger populations and additional morphokinetics.
Wider implications of the findings: This is the first observation thoroughly describing the development of embryos derived from *in vitro* matured MI oocytes obtained from COH. MI-derived embryos lower the fertilization rate and significantly slower the morphokinetics than that of MII oocytes. Therefore, ovarian stimulation should be performed carefully for mature oocytes obtained at retrieval.
Trial registration number: We don't need to trial registration number.

P-170 Visualization of the metaphase II meiotic spindle and effect on the rate of multiple pronuclear formation in ART patients with positive anti-centromere antibodies

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Study question: Do anti-centromere antibodies (ACA) have an effect on the presence of the meiotic spindle and the rate of multiple pronuclear (MPN) formation in human embryos?
Summary answer: The present results suggest that ACA are the cause of the high rate of MPN formation due to an inhibition of mitotic spindle formation.
What is known already: ACA is an anti-nuclear antibody (ANA), and specifically recognizes the centromere. Recently, several studies have reported that the rate of MPN formation is higher in patients with ACA. We previously documented that in MI oocytes collected from ACA patients, the female chromosome were frequently dispersed in the cytoplasm. This abnormality of the female chromosome may be a cause of MPN formation. However, the association between the formation of the meiotic spindle and the high rate of MPN formation in ACA patients needs to be further investigated.
Study design, size, duration: Between May 2013 to April 2015, 160 MII oocytes from 18 treatment cycles in 8 patient cases (ACA group) found to be ACA positive following testing and 2632 MII oocyte from 362 cycles in 294 patient cases (Control group) which was not tested for ACA after oocyte retrieval from February to August 2008 were analyzed.
Participants/materials, setting, methods: The spindles of MII oocytes were examined by the the OosightTM imaging system (OIS). We compared the presence of the spindle (presence or absence) of ACA and control groups. After ICSI, the rate of MPN in ACA and control groups were recorded. MPN embryos were stained with H3K9me2 antibody to detect the female chromosome.
Main results and the role of chance: The rate of incidence of a visible spindle in ACA oocytes was significantly lower than that found in controls (38.1% versus 89.2%; $P < 0.01$). The rate of MPN formation after ICSI in ACA (visible spindle: 26.2%, no visible spindle: 60.6%) was significantly higher than that found in control embryos (visible spindle: 2.3%, no visible spindle: 7.4%) ($P < 0.01$). In MPN embryos originating from ACA cases, 100% (52/52) of the MPN embryos following ICSI had a single male PN and 2 or more female PN.
Limitations, reasons for caution: Retrospective analysis with a limited sample size due to rarity of condition.
Wider implications of the findings: The present results suggest that the female chromosome is frequently dispersed in the cytoplasm of MI oocytes collected from ACA patients. It seems highly likely that this abnormality is the direct cause of MPN formation.
Trial registration number: None.

P-171 Morphokinetic behavior of chromosomally normal and abnormal embryos in patients under 36 years old undergoing PGS for aneuploidy screening

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Study question: Can time-lapse monitoring of preimplantation embryo development pattern be uniformly defined in order to select noninvasively euploid embryos in patients under 36 years of age?
Summary answer: Late first cleavage, late PN fading and longer transition period between morula-early blastocyst stage were observed in aneuploid embryos as compared to euploid embryos.
What is known already: It is known that embryo aneuploidy is a major cause of IVF failure, however, PGS is not always possible due to legal, economical or sociocultural reasons. Aneuploidy has been correlated with specific morphokinetic variables used previously to develop an aneuploidy classification.

Irregular division pattern, delays in the first cell cycle and asynchrony in the disappearance of the pronuclei in abnormal embryos had previously been reported. However, some studies failed to confirm these findings. Moreover, late compaction and/or blastulation initiation and completion delay in aneuploid embryos as compare to euploid embryos has been reported.
Study design, size, duration: Embryo development was retrospectively analyzed using time lapse system EmbryoScope™ (Unisense, Danmark) in a total of 163 embryos, scheduled for aneuploidy screening with aCGH in patient of under 36 years old. These embryos were monitored in a special tri-gas incubator. The morphokinetic characteristics of each embryo were monitored until day 5.

The data were collected between August 2015–December 2015. The study was approved by ethical committee of IVI.
Participants/materials, setting, methods: Embryo biopsy was done on D3 of embryo development and chromosome screening performed through array-CGH. Development of these embryos had been monitored in a controlled environment. Images were captured in every 20 minutes from 7 different focal planes. The analyzed parameters were t2 to tSB, cc2 (t3-t2), S2(t4-t3) and S3(t8-t5). T test was used for comparison of mean timings. *P*-value < 0.05 has been considered statistically significant.
Main results and the role of chance: The mean age of our female population included in the study was 32.5 years. The indication for PGS was repeated implantation failure, described as more than 2 failed previous cycles.

Among 163 embryos analyzed, 55 had normal chromosomal content and 37 embryos were transferred in 25 cycles (mean of 1.5 embryos/cycle).

Interestingly, the results suggest that there was a statistically significant difference in tPNf ($p = 0.0432$), t2 ($p = 0.0114$), and tSB-tM ($p = 0.0044$) between abnormal and normal PGS results.

Controversially, no statically significant differences were found between euploid and aneuploid embryos for the rest of the morphokinetics parameters; tPB2, tPNF-tPB2, t3, CC2, t4, S2, t5, t6, t7, t8, t9+, tM, tSB and tM-T9.
Limitations, reasons for caution: Associations between morphokinetics and ploidy require careful interpretation due to lack of consensus for definitions, variable PGS methodologies, sample sizes and time lapse devices used. Larger studies are needed but time lapse imaging remains a promising tool to enhance clinical outcome.
Wider implications of the findings: The selection of the embryos through time-lapse technology should not be considered as a replacement for PGS. However, there is a clear benefit gained from the morphokinetics screening and selection according to these findings.

Further prospective studies are needed in order to clinically validate these results.
Trial registration number: Not applicable.

P-172 First pregnancies from human embryos vitrified-warmed using the semi-automated Gavi closed vitrification system

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Study question: Are clinical outcomes from the transfer of human blastocysts vitrified using the semi-automated closed Gavi™ system equivalent to that of the manual open Cryotop™ system?
Summary answer: Preliminary clinical outcomes of blastocysts vitrified using the Gavi systems are comparable to the Cryotop system including embryo recovery, embryo survival and biochemical pregnancy rates.
What is known already: Vitrification of embryos is an essential component of an effective assisted conception program. When successfully executed, it

provides excellent embryo survival and pregnancy rates, with the gold-standard method arguably being the open Cryotop system. However, vitrification is a manual, high-skill and labor intensive procedure that is difficult to standardize, and results can vary greatly between embryologists and clinics. To address these issues, we developed the Gavi system, a semi-automated closed vitrification system. *In vitro* studies using mouse and human research blastocysts have shown equivalent results to the Cryotop system (Roy et al., 2014).

Study design, size, duration: This is an ongoing pilot study in which infertile pre-implantation genetic screening (PGS) patients were randomly allocated to have either their best embryo for cryopreservation vitrified using the Gavi system and their second best using the Cryotop system, or vice versa. This study, which commenced in August 2015, will assess outcomes of 50 single embryo transfers from both Gavi and Cryotop vitrified-warmed blastocysts. Results presented below are interim results from the ongoing study.

Participants/materials, setting, methods: This study was performed at the private assisted reproductive technology (ART) clinic Genea (Sydney, Australia), which has proven expertise in Cryotop vitrification (Roy et al., 2014). Participants included infertile couples undergoing assisted conception and PGS with at least one blastocyst for vitrification. Vitrification was performed using either Gavi, as described by the manufacturer, Genea Biomedx, or the open Cryotop protocol as described previously (Roy et al., 2014).

Main results and the role of chance: 180 blastocysts have been vitrified using the Gavi system and 190 vitrified using the Cryotop system. Twenty euploid embryos vitrified using the Gavi system and 15 using the Cryotop system have been warmed, all of which were recovered from the vitrification device and survived the warming process as defined by greater than 70% cells intact. A single embryo transfer policy followed and the average maternal age of Gavi vitrified-warmed embryos was 36.0 years and 35.9 years for Cryotop vitrified-warmed embryos. Of the vitrified-warmed embryos transferred 85% of Gavi system embryos were of excellent quality (ICM and TE grade excellent or good) prior to vitrification as compared with 80% of Cryotop embryos. Clinical use of Gavi vitrified-warmed embryos resulted in a 64.7% (11/17 βhCG positive, 3 pending) biochemical pregnancy rate as compared with 83.3% (10/12 βhCG positive, 3 pending) for Cryotop vitrified-warmed embryos ($p = 0.27$). Of the biochemical pregnant patients that have undergone ultrasound at 7 weeks, 100% (9/9, 2 pending) of Gavi system pregnancies had a fetal heart beat as compared with 89% (8/9, 1 pending) of Cryotop system pregnancies. The first pregnancy using a Gavi vitrified-warmed blastocyst is now at 16 weeks and progressing normally.

Limitations, reasons for caution: The main limitations of this study are (1) small sample size and (2) the absence of live birth outcomes. Clinical outcomes of Gavi-vitrified cleavage stage embryos or oocytes were not assessed.

Wider implications of the findings: The Gavi system has the potential to revolutionize embryo vitrification; standardizing the process, increasing laboratory efficiencies and improving clinical outcomes for laboratories struggling with cryopreservation. Additionally, it would show it is possible to semi-automate complicated ART procedures and open up the possibility for further improvements in efficiencies and clinical outcomes.

Trial registration number: Not registered.

References

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P-173 Does automatic time-lapse embryo test improve embryo selection based on morphology?

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Study question: Is there a correlation between the automatic classification provided on Day 3 by the Eeva (Early embryo viability assessment) system (High, Medium or Low) with implantation rates (IR)?

Summary answer: There is a direct correlation according to the automatic test embryo score, improving morphological score, being useful for the embryo selection process.

What is known already: Wong et al. (2010) developed an algorithm to classify embryos according to their probability of reaching blastocyst stage based on the combination of two parameters: P2 = t3–t2 and P3 = t4–t3 which are automatically calculated by time-lapse cell tracking. VerMilyea et al. (2014) showed that embryos with High and Medium scores had significantly higher implantation rates than those with Low scores. This test has been performed in five different clinics in the USA (Conaghan et al., 2013) showing an improvement on embryo selection when Eeva classification was used and a decrease in variability among embryologists.

Study design, size, duration: Retrospective cohort study, from October 2013 to December 2015.

Participants/materials, setting, methods: University-affiliated infertility clinic. The study includes 436 cycles in which embryos were incubated in a conventional incubator with especially designed scopes that used an automatic cell-tracking software to analyze the exact timing of first and second cleavages. The system provided a classification according to P2 and P3. From these cycles a total of 784 embryos were transferred.

Main results and the role of chance: We observed a direct relationship between morphokinetic categories and implantation potential. Results were only referred to those embryos with known implantation ($n = 521$).

When categorizing according to EEVA: 158 embryos were labelled as High, 114 as Medium and 249 as Low. Implantation rates in each of the categories were: HIGH 46.80%; MEDIUM 36.80%; LOW 27.70%. We observed this same correlation when distinguishing the day of transfer: DAY3: HIGH 38.20%; MEDIUM 31.70%: LOW 26.10%. For DAY5 transfer IR were: HIGH 66.70%; MEDIUM 50% and LOW 31%.

A multi-variable analysis was performed to quantify the chances of implantation when a high embryo is transferred and considering potential bias factors such as oocyte quality or day of transfer (Table 1). A predictive model was developed comparing predictive properties of ASEBIR morphology selection vs selection based on Eeva categories for implantation (0.622 and 0.650 respectively), indicating the moderate utility of both selection methods but showing a better predictive value of Eeva test.

		OR	CI 95%	
EEVA Categories	MEDIUM vs LOW	1.37	0.835–2.248	ns
HIGH vs LOW	2.238	1.441–3.475	>0.001	
ASEBIR (morphology) Categories	B vs C	1.062	0.529–2.133	ns
A vs C	1.45	0.791–2.657	ns	
	Donated vs Own oocytes	3.174	1.505–6.696	>0.001
	Blastocyst vs Cleavage stage	2.171	1.436–3.282	>0.001

Limitations, reasons for caution: The retrospective nature of this study may be a reason for caution; nevertheless, it is the first step previous to future prospective studies. The classification system itself has some errors due to difficulties in cell tracking generating "none result", however we only included cases where a classification was provided

Wider implications of the findings: Our study has demonstrated that embryo selection by automated time-lapse supported by the use of a two variable morphokinetic model is related with reproductive outcome. These results also show that embryo morphology together with Eeva categorization slightly increase predictive properties to select embryos with higher implantation potential.

Trial registration number: None.

P-174 Clinical outcomes after use of Embryo-Glue as a human embryo transfer (ET) medium in warming cycles

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Study question: To evaluate the efficacy of Embryo-Glue as a human ET medium for warming cycles.

Summary answer: Embryo-Glue as ET medium for warmed embryos is as effective as the routine medium for infertile patients undergoing warming cycles.

What is known already: Hyaluronan (HA) is a major glycosaminoglycan present in follicular, oviduct and uterine fluid. HA concentration increases at the time of implantation. Embryo-Glue (EG) is a ready use medium that contains a high concentration of hyaluronan (0.5 mg/mL). EG has an implantation-promoting

effect in fresh IVF cycles in selected patients such as patients with poor quality embryos, repeated implantation failure and advanced maternal age.

Study design, size, duration: To validate EG usefulness in our laboratory, a prospective randomized study was carried out between June 2015 and January 2016 including a total of 253 patients undergoing 328 warming cycles. Embryo transfers were performed either in Embryo-Glue ($n = 136$) as study group or in routine ET medium as control group ($n = 192$).

Participants/materials, setting, methods: A total of 372 warmed and transferred embryos were included in the study. After warming, the embryos were cultured for 24 hours and transferred if cleaved (Embryo-Glue $n = 155$ vs ET conventional medium $n = 217$). The embryos were prospectively randomized using different lists according to the developmental stage (Day2/Day3 and Day5/Day6) between the two groups.

Main results and the role of chance: Patients' characteristics were similar for both groups in terms of maternal age, number/quality of embryos transferred, endometrial preparation and degree of difficulty of embryo transfer. Global results on the 372 transferred embryos did not showed significant differences between two groups. Pregnancy rate per transfer were (29.4% vs 23.9% $p = 0.32$) and biochemical and abortion rates were (5.1% vs 8.3% $p = 0.37$; 2.2% vs 5.2% $p = 0.32$ respectively). For Day2/Day3 embryos transfers the pregnancy rate per transfer were 22.7% ($n = 97$) vs 18.8% ($n = 143$) for EG and the control group, respectively. For Day5/Day6 embryos transfers the difference seems to in favor of EG: 46.1 ($n = 39$) vs 38.8% ($n = 49$), albeit not statistically significant.

Limitations, reasons for caution: The study was performed on unselected patients based on a prospectively randomized list. However, the two groups had similar characteristics. These are preliminary results and the small number actually included may impair the significance of the results. The comparison is still ongoing to increase the number.

Wider implications of the findings: High concentration of HA in ET medium displayed high pregnancy rate for warming cycles validating the use of EG in the laboratory. However, the difference in PR between the two groups prompts us to carry on the study in order to confirm the eventual beneficial effect of EG.

Trial registration number: None.

P-175 Mitochondrial DNA content as a measure of implantation potential in human euploid blastocysts: correlation with embryo quality and female age

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Study question: Is mitochondrial DNA content correlated to female age and embryo quality, or provides an independent measure of embryonic implantation potential?

Summary answer: According to our results, mitochondrial DNA content provides a useful biomarker of embryonic implantation potential, irrespective of embryo quality and female age.

What is known already: A potential association between high quantities of mitochondrial DNA (mtDNA) and the failure of human embryos to implant has been recently described. In particular, the incorporation of mtDNA copy number analysis into the routine preimplantation genetic screening (PGS) has been proposed, in order to select the euploid blastocysts with the best chances to implant.

To date it is unclear whether this new biomarker provides an independent score of embryo viability, irrespective of standard assessments, such as embryo quality, or female age: in literature, few and conflicting data appear on these issues.

Study design, size, duration: This is a retrospective observational study performed between October 2013 and January 2015. The study included the data analysis of 54 euploid blastocysts obtained from 26 patients (average age 35.5 years, range 26–42 years) following 29 PGS cycles. Single embryo transfers of 43 euploid blastocysts were performed.

The relative amount of mtDNA was assessed in relation to implantation potential, blastocysts quality and female age.

Participants/materials, setting, methods: Blastocysts quality was assessed and categorized in two groups, according to morphology and expansion (good and poor quality).

Trophectoderm biopsy was undertaken on Day 5–6 and comprehensive chromosome screening (CCS) performed through array-CGH.

Part of whole genome amplification products, necessary for CCS, was subjected to real-time PCR to quantify the mtDNA content for each of 54 euploid blastocysts. Blastocysts were categorized in two groups, according to a relative mtDNA threshold value (0.003) described in literature.

Main results and the role of chance: Twelve out of 54 euploid blastocysts showed high mtDNA levels (22.2%). The remaining 42 showed low mtDNA levels.

mtDNA content and implantation potential

Out of 43 transferred blastocysts, 11 resulted in pregnancies and successfully gave 11 healthy live births. All the pregnancies (100%) originated from blastocysts with low mtDNA levels. None of the blastocysts with high mtDNA quantities led to pregnancy.

mtDNA content and blastocysts quality

Out of 54 euploid blastocysts, 19 were classified as "poor quality blastocysts": 5 (26.3%) had high mtDNA levels and 14 (73.7%) showed low mtDNA quantities. Thirty five were classified as "good quality blastocysts": 7 (20%) had high mtDNA levels and 28 (80%) showed low mtDNA quantities. Statistical analysis revealed no correlation between embryo quality and mtDNA content inside each group.

mtDNA content and female age

The mean age of the patients with high mtDNA levels blastocysts was 35.4 years (range 30–42 years). The group of patients with low mtDNA quantities blastocysts showed a mean age of 35.7 years (range 26–42 years). No statistical significant difference, in terms of female age, was found between blastocysts with high and low mtDNA content.

Limitations, reasons for caution: Limited number of cases of the study group.

Relatively low average age of the patients (35.6 years), with no patients above 42 years of age: it would be interesting to evaluate the mtDNA content of euploid blastocysts in older patients.

Wider implications of the findings: This study provides further support for the relationship between mtDNA quantity in trophectoderm cells and embryo implantation potential and demonstrated that mtDNA content evaluation provides an independent score of embryo viability, irrespective of embryo quality and female age. Therefore, the inclusion of mtDNA analysis into routine PGS should be considered.

Trial registration number: None.

P-176 The effect of sperm selection with fertile plus on intracytoplasmic sperm injection outcome

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Study question: The aim of the current study is to evaluate to effectiveness of sperm selection by using fertile plus chip on intracytoplasmic sperm injection (ICSI) cycles.

Summary answer: Fertilization rate, percentage of grade 1 embryo numbers and pregnancy rates were similar between sperm selection with fertile plus chip and classical gradient technique.

What is known already: Selection of sperm in *in vitro* fertilization (IVF)/intracytoplasmic sperm injection (ICSI) has a key role in the success. Fertile plus chip is chemical-free microchips that mimic natural paths of sperm, effective way of enabling selected motile sperm. Sperm selections with fertile plus provides less oxidative stress and less DNA damage.

Study design, size, duration: This retrospective analyze was constituted with the patients attended and underwent ICSI program at the Assisted Reproduction Unit of Baskent University between September 2014 and January 2016. Clinical data were obtained from patients' records. The study eligibility criteria were as follows: women younger than 40 years old and couples who had one or more IVF failure. In the 549 cycles gradient technique and in 102 cycles fertile plus chip were used for sperm selection.

Participants/materials, setting, methods: Data are expressed as means ± SD. The baseline differences between the two groups were analyzed by Student's

t test. A two sided p value < 0.05 was considered statistically significant. Data were analyzed with the use of SPSS for Windows (version 17.0; SPSS Inc., Chicago, IL). In contingency tables, the Chi-Square test was performed.
Main results and the role of chance: Baseline characteristics of the patients (the mean age of patients (33.21 ± 4.96 vs. 33.43 ± 4.43), the duration of infertility (6.03 ± 4.16 vs. 6.17 ± 3.88) were not significantly different between the groups. Metaphase II oocyte number (7.33 ± 6.74 vs. 9.15 ± 7.31), fertilization rate (70.27 ± 52.59 vs. 67.93 ± 21.91), grade 1 embryo numbers (0.69 ± 0.78 vs. 0.80 ± 0.80), transfer day (3.72 ± 1.01 vs. 3.74 ± 1.04), and transferred embryo numbers (1.67 ± 0.48 vs. 1.71 ± 0.46) were also similar in the two groups. Pregnancy rates were 38.4% in classical gradient technique group and 38.2% in the fertile plus chip group and the difference was not statistically significant.
Limitations, reasons for caution: The major limitation of our study is that the data were collected retrospectively. There are two different microfluidic design for sperm sorting. We use fertile plus for ICSI. These results cannot be said for fertile. Our another prospective randomized double blind study (ClinicalTrials.gov Identifier: NCT02488434) has been continued.
Wider implications of the findings: To our knowledge this is first clinical trial about fertile plus use for ICSI. This study didn't find any difference in terms of fertilization rate, embryo quality and pregnancy rate. But further studies may demonstrate molecular differences.
Trial registration number: This study is retrospective so we didn't obtain registration number.

P-177 Are double embryo transfers' outcome influenced by the quality of the second embryo?

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Study question: Has the quality of the second embryo in double transfers impact in the success of ART cycles?
Summary answer: Our data suggest the quality of the second embryo in double embryo transfers (DET) has no influence in the success of ART cycles.
What is known already: There is little literature concerning the impact of DET regarding different quality embryos' inter-influence. There is no consensus about the overall pregnancy rates differing or not significantly whether a poor-quality embryo is added to the transfer of one good-quality embryo. However, twining rates seem to be significantly different between the transfers of two top-quality embryos, and one top and one poor. It is known that multiple pregnancies increase their associated maternal and perinatal risks. Therefore, double embryo transfer should be thought with some concern, particularly in young patients.
Study design, size, duration: Retrospective analysis of data regarding 655 IVF or ICSI cycles, performed between October 2008 and December 2014, using agonist long protocol with DET and the transfer of at least one top quality embryo. We classified the embryos on day 2 or 3, taking into account multiple morphological criteria (cell number, degree of cytoplasmatic fragmentation, multinucleation, cell size regularity, presence of vacuoles, etc), in four categories (A, B, C, D), based on ASEBIR classification.
Participants/materials, setting, methods: The study population was <40 years old. Taking into account that at least one top quality embryo was transferred, cycles were divided into four groups. Group I (n = 296) contains two grade A embryos, Group II (n = 212) one A and one B, Group III (n = 111) one A and one C, and Group IV (n = 36) one A and one D. Clinical pregnancy, multiple pregnancy, miscarriage, live birth, preterm, duration of gestation and birth complications rates were evaluated.
Main results and the role of chance: The overall clinical pregnancy rate per transfer in our clinic during that period was 44.5%. In this study the rates were 56.1%, 55.2%, 52.3% and 41.7% for Group I, II, III and IV respectively. It was visible that there was a difference in clinical pregnancy rate per transfer between Group I and Group IV, but it was not statistically significant. There was no difference in multiple pregnancy rate among all groups (18.9% vs 13.7% vs 18.0% vs 13.9%). Miscarriage rate was lower (6.7%) in group IV when compared with the rest of the study groups (15.7%; 20.5%; 24.1%), but this was also not statistically significant. Live birth rates per transfer were 45.6% vs 41.9% vs 36.9% vs 33.3% with no statistically significant differences among the four groups. The analysis of preterm delivery rate, duration of gestation and birth complications revealed no differences in all study groups too. Women's age was similar in the four groups.
Limitations, reasons for caution: This study is limited by its retrospective nature and sample size. Also, the four study groups have a very different size. Results must, therefore, be considered with some caution.
Wider implications of the findings: Our results suggest that, in our working conditions, there has neither been an embryo-helping nor embryo-detrimental effect in double transfers. This information should be considered in the counseling of patients concerning DET vs SET.
Trial registration number: Not applicable.

P-178 Influence of sperm quality on the development to blastocyst stage and its ploidy status

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Study question: Does the sperm quality correlate with the ploidy status of the derived blastocysts?
Summary answer: Blastocyst derived from poor quality sperm have the same probability to be euploid compared to ones formed from normal semen.
What is known already: Thanks to the ICSI, in severe male factor infertility it becomes possible to offer IVF treatments, although extremely impaired sperm quality may have an impact on fertilization rates. Several study analyzing the clinical outcomes obtained with embryos derived from normal sperm samples compared to severe sperm morphology abnormalities, report very conflicting data. High rate of DNA fragmentation, mitochondrial dysfunction and chromosomal aneuploidy seem to be significantly higher in the spermatozoa from oligozoospermic and/or asthenozoospermic and/or teratozoospermic (OAT) males compared with unaffected controls. However, to date few data regarding the impact of the sperm quality on embryogenesis are reported.
Study design, size, duration: This cohort retrospective study was performed from January 2014 to December 2015 on 148 Preimplantation Genetic Screening (PGS) cycles. The genetic outcome of 586 blastocysts were analyzed by mean of Whole Genome Amplification (WGA) and array-Comparative Genomic Hybridization (a-CGH). All mature oocytes retrieved were injected and cultured individually until the blastocyst stage at 37°C, 6%CO2, 5%O2. All biopsies were performed at blastocyst stage. Mean female and male ages were 34.95 ± 4.5 and 38.05 ± 5.9 years old, respectively.
Participants/materials, setting, methods: All the patients enrolled in this study submitted to the following selection criteria: maternal age ≤36 years old, only standard ICSI to be performed, normal karyotype of both parents, genetic diseases and female infertility factors were excluded. All cycles were divided in 4 groups on the basis of semen parameters performed according to WHO 2010: group 1 normal; group 2 OAT; group 3 severe OAT; group 4 NOA (Non-Obstructive Azospermia) and testis cancer patients.
Main results and the role of chance: The number of cycles enrolled were 88, 53, 20, 18 in groups 1, 2, 3, 4, respectively. The number of oocytes injected were 775, 508, 180, 184 in groups 1, 2, 3, 4, respectively. There was no difference in fertilization rate between groups 2, 3, 4: 66.1% (336/508), 66.1% (119/180), 55.5% (120/184), respectively; a statistically higher (p < 0.01) fertilization rate was found in group 1: 77.2% (598/775). The number of embryos obtained were 598, 336, 119, 120 in groups 1, 2, 3, 4, respectively. The blastocysts obtained were: 414 (69.2%), 241 (71.7%), 71 (59.7%), 62 (51.7%), respectively. The apparently lower blastocyst formation rate found in the group 4 compared to the other 3 groups does not reach a statistical significance, probably due to the low number of cycles enrolled in this group. Surprisingly, the rate of euploid/biopsied blastocysts was the same in all groups, even in number 4 in which sperms are recovered from testis: 45.5% (140/308), 53.8% (99/184), 58.6% (34/58) and 55.6% (20/36) in groups 1, 2, 3, 4, respectively. Pregnancy rates were 59.0% (46/78), 66.7% (30/45), 62.5% (10/16), 64.3% (9/14) in groups 1, 2, 3, 4, respectively (NS).
Limitations, reasons for caution: The number of cycles eligible to be included in the study is still quite low, due to the strict inclusion criteria established in order to select male factor only patients; therefore, further cycles need to be

performed to confirm these preliminary results. For that reason, the study is still ongoing.
Wider implications of the findings: Our data gives strong hope to men with an extremely low semen quality. Due to the poor fertilization rate expected, it is advisable to increase the number of oocytes to inseminate and to extend the culture until the blastocyst stage since blastocysts euploidy probability is independent from the semen used.
Trial registration number: Not applicable.

P-179 Chromosomic abnormalities involved in complex aneuploidies in human blastocysts after *in vitro* fertilization and trophoectoderm biopsy for Preimplantation Genetic Screening (PGS) cycles

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Study question: This study evaluates chromosomes involved in complex aneuploidy (CA) in human blastocysts after PGS cycles performed with trophoectoderm biopsy and array comparative genomic hybridization (aCGH).
Summary answer: Chromosomes mainly involved in CA are 16-21-22 and there is an increasing in the percentage of abnormalities of these chromosome with increasing maternal age.
What is known already: Among the main causes of infertility and failure of Assisted Reproduction Technique (ART) cycles are aneuploidies in embryonic genome. Chromosomal aberrations are simple (monosomies-M or trisomies-T) or complex (2 or more chromosomes involved). Aneuploidies rates are strictly correlated with maternal age because in women older than 40 years old about 80% of the oocytes presents aneuploidies due to meiotic non-disjunction. Some chromosomes are more prone than others to develop aneuploidies caused by non-disjunction events. These chromosomes are 16, 21, 22 due to their size and morphological structure: they are all small chromosomes and 21 and 22 have an acrocentric centromere.
Study design, size, duration: From September 2011 to September 2015, 977 PGS cycles were performed, and 3143 blastocysts were analyzed after trophoectoderm biopsy and aCGH. After genetic results, CA were analyzed to investigate the percentage of aneuploidies (monosomies and trisomies) for each chromosome and to determine different trends in the distribution of the aneuploidies among all chromosomes. CA were considered to study the percentage of aneuploidies in different chromosomes as the data were more consistent.
Participants/materials, setting, methods: Patients were divided according to maternal age in 4 groups: ≤30, 31–35, 36–40 and ≥41 years old in group A, B, C, D where 42, 209, 445 and 281 cycles were performed, respectively. All mature oocytes retrieved were injected and cultured individually until the blastocyst stage at 37°C, 6%CO2, 5%O2. After trophoectoderm biopsy, blastocyst genetic asset was anlyzed by Whole Genome Amplification (WGA) followed by aCGH.
Main results and the role of chance: Analyzed blastocysts were 180, 864, 1473, 626 in group A, B, C, D, respectively. Aneuploid embryos dramatically increase with maternal age ($p < 0.001$) and CA are higher in group-D, compared with groups A, B, C ($P < 0.001$). In group-A, 97 (53.9%) blastocysts resulted euploid, 15 (8.3%) mosaic, 3 (1.7%) no-results and 65 (36.1%) aneuploid. Among aneuploid embryos, 35 (53.8%) were CA, 20 (30.8%) M and 10 (15.4%) T. In group-B, 401 (46.5%) blastocysts resulted euploid, 70 (8.1%) mosaic, 22 (2.5%) no-results and 371 (42.9%) aneuploid. Among aneuploid embryos 201 (54.2%) were CA, 102 (27.5%) M and 68 (18.3%) T. In group-C, 536 (36.4%) blastocysts resulted euploid, 90 (6.1%) mosaic, 34 (2.3%) no-results and 813 (55.2%) aneuploid. Among aneuploid embryos, 421 (51.8%) were CA, 221 (27.2%) M and 171 (21%) T. In group-D, 90 (14.4%) blastocysts were euploid, 35 (5.6%) mosaic, 8 (1.3%) no-result and 493 (78.7%) aneuploid. Among aneuploid embryos, 361 (73.2%) were CA, 53 (10.8%) M and 79 (16%) T. Most involved chromosomes in CA were: 1 (5.7%), 16 (7%), 18 (5.7%) in group-B; 16 (7%), 21 (6.7%), 22 (7.4%) in group-C and 16 (6.8%), 21 (6.9%), 22 (7.9%) in group-D, respectively. In group-A no differences were found regarding all chromosomes.
Limitations, reasons for caution: The influence of male age in chromosomic aberration should be considered, since it is reported that in embryos from couples with men older than 50 years old the aneuploidies in some chromosomes are higher than in embryos from couples with men younger than 50 years old.
Wider implications of the findings: Genome of degenerated embryos could be analyzed to find if aneuploidies in other chromosomes than 16, 21 and 22 are really less represented or if they could influence the early stages of embryo development, leading to degeneration before reaching the blastocyst stage.
Trial registration number: Not applicable.

P-180 Endometrial injury and the quality of embryos are the most important prognostic factors for in-vitro-fertilization success after previous repeated unsuccessful attempts.

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Study question: Which are the most important prognostic factors for achieving a pregnancy after in-vitro-fertilization (IVF) in women with history of at least three consecutives unsuccessful IVF attempts (RIF)?
Summary answer: Endometrial injury, the quality of transferred embryos and transfer of blastocyst stage embryos are the most important factors affecting pregnancy rate in women with RIF.
What is known already: Repeated IVF treatment failure (RIF) is very frustrating to the patients and to the clinicians. It may occur due to a variety of reasons, but reduced endometrium receptivity and low embryo quality are thought to be the most important. Several approaches have been proposed to improve success rate in these women and amongst others include local endometrial injury (LEI) and blastocyst transfer. There is some evidence in the literature to support these approaches, however, in unselected group of women with RIF, LEI together with other prognostic factors affecting pregnancy rate has not yet been studied using multiple logistic regression model.
Study design, size, duration: A retrospective study including 429 IVF/ICSI cycles that was performed at the Department for reproductive medicine, University Medical Centre, Maribor. Study period was from January 2013 to December 2014.
Participants/materials, setting, methods: Women younger than 40 years with at least three previous consecutive failed IVF attempts were included. They underwent controlled ovarian stimulation and IVF/ICSI procedure. According to the doctor-patient agreement some women had hysteroscopy with local endometrial injury (LEI) in the preceding cycle. Patient's and cycle's characteristics were compared between the conception and non-conception cycles. The univariate and multivariate logistic regression model was used to identify the most important prognostic factors for clinical pregnancy.
Main results and the role of chance: Clinical pregnancy was observed in 140 (32.6%) cycles. There were no statistically significant differences between conception and non-conception cycles in women s age (33.82 ± 3.41 vs. 34.52 ± 3.07), number of previous cycles (4.30 ± 1.44 vs. 4.75 ± 2.23), causes of infertility and number of transferred embryos (1.89 ± 0.49 vs. 1.79 ± 0.65). Statistically significant different total dosage of gonadotrophins (28.82 ± 10.21 vs. 31.21 ± 11.68), number of oocytes retrieved (11.61 ± 5.19 vs. 9.93 ± 5.79), number of embryos (6.95 ± 3.78 vs. 5.34 ± 3.52), number of good quality embryos on day two (4.63 ± 3.63 vs 3.35 ± 3.12), number of blastocysts (2.76 ± 2.90 vs. 1.47 ± 2.46), number of freezing blastocysts (1.46 ± 2.13 vs. 0.80 ± 1.71), proportion of cycles with embryo freezing (49.29 vs. 32.53), proportion of cycles with transfer of at least one good quality embryo (76.43 vs.56.75), proportion of cycles with day five transfer (50.71 vs 30.43) and proportion of women with LEI (28.57 vs. 17.75) was observed in conception compared to non-conception cycles. These parameters were also found to be associated with clinical pregnancy using univariate logistic regression. In multivariate logistic regression model only transfer of at least one good quality embryo (OR: 4.32, 95% CI: 2.41–7.73), transfer on day five (OR: 3.02, 95% CI: 1.53–5.94 and LEI (OR: 1.73, 95% CI: 1.02–2.92) remained important independent prognostic factor for clinical pregnancy.
Limitations, reasons for caution: This is a single-centre retrospective analysis and despite robust methodological approach, the presence of potential bias cannot be excluded. The proportion of patients with LEI is relatively low.
Wider implications of the findings: Results of our study suggest that transfer of blastocyst stage embryos and hysteroscopy with local endometrial injury should be recommended in patients with repeated IVF failure in order to

improve the pregnancy rate. Larger prospective multicentric studies are needed to confirm these findings.
Trial registration number: /

P-181 Identification of candidate epigenetic regulators that can be used to examine and monitor the epigenetic impact of assisted reproductive technologies (ART)

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Study question: Can expression of key epigenetic regulators provide predictive value to detect adverse conditions resulting from ART practices?
Summary answer: Expressions of *Dnmt3b* and *Prdm14* are sensitive to suboptimal conditions examined and can be used to monitor the epigenetic impact of ART procedures and materials.
What is known already: Growing concerns for epigenetic effects of ART procedures have been raised by the observation of an increased number of rare genetic disorders in children born after ART. Two general classes of DNA methyltransferases—de novo methyltransferases (DNMT3a and 3b) and maintenance methyltransferase (DNMT1)—were shown to have dynamic expression patterns during pre-implantation. A PR-domain-containing transcriptional regulator (PRDM14) and ESRRB were recently found to regulate DNMTs and epigenetic pathway. Incorporating evaluations of epigenetic regulator expression in an assay for screening materials and conditions could benefit risk assessment and help understand the link between epigenetic disorders and ARTs in mammals.
Study design, size, duration: Expression of known epigenetic regulators—*Dnmt1, Dnmt3a, Dnmt3b, Prdm14, Esrrb*—as candidate markers were evaluated with immunocytochemistry (ICC) from different stages of mouse embryo development (oocyte, 2-cell, 4-cell, 8-cell, morula, blastocyst, expanded/hatching blastocyst) cultured under optimal (control oil, n = 166) or suboptimal (7.5% toxic oil, n = 166) conditions for 96hrs. Experiments were repeated more than three times.
Participants/materials, setting, methods: One-cell mouse embryos (from frozen) were cultured uninterrupted up to 96 hours in Continuous Single Culture Medium-Complete (CSCM-C, Irvine Scientific) with control or suboptimal oil overlay. Embryos were fixed at T = 0, 24, 36, 48, 72, and 96 hrs for ICC, and incubated with primary antibody, then secondary fluorescent antibody to detect expression of epigenetic genes. Sox2 expression was detected as a control. Blastocyst rates (%) were evaluated at 96 hrs (n = 23 to 28).
Main results and the role of chance: The embryos cultured with suboptimal oil overlay showed a noticeable delay in development (at 72 hrs and 96 hrs compare to the control oil group) as expected. Compromised expression of DNMT3b and PRDM14 was observed in embryos cultured with suboptimal oil overlay, whereas no significant difference in expression of DNMT1, DNMT3a, and ESRRB was observed between embryos in the suboptimal oil group vs. control group. Of the five candidate genes examined, our studies revealed that DNMT3b and PRDM14 could serve as two candidate epigenetic regulators which can potentially be used to detect adverse culture conditions and further evaluate epigenetic effects.
Limitations, reasons for caution: Since embryos are inherently different from one another, each embryo may respond to the toxicity differently making observations difficult. Detection of gene expression depends largely on the performance of antibodies and reagents used in ICC.
Wider implications of the findings: Our results indicate that epigenetic regulators can be used as early biological markers to examine the impact of suboptimal conditions during pre-implantation mammalian embryo development. With the heightened sensitivity, the functional impact of materials and procedures on overall embryo development including embryo health and epigenetic defects can be adroitly evaluated.
Trial registration number: N/A.

P-182 Effective and patient-friendly endometrial preparation method for frozen-thawed embryo transfer in patients with polycystic ovary syndrome: ovulation induction with letrozole

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Study question: Which is better for endometrial preparation method in frozen-thawed embryo transfer (FET) cycles, hormone replacement therapy (HRT) or ovulation induction with letrozole?
Summary answer: FET after ovulation induction with letrozole showed significantly higher ongoing pregnancy rates than FET after HRT in patients with polycystic ovary syndrome (PCOS)
What is known already: Frozen-thawed embryo could be transferred in natural cycle and HRT cycle. FET in natural cycle has several advantages like avoiding medication and painful injection. However, in case of patients with PCOS, embryos are usually transferred in HRT cycle due to menstrual irregularity. Letrozole gains popularity for ovulation induction in PCOS patients. It usually promotes single follicle development and exhibits no negative effect on endometrium. Therefore, it could be used for endometrial preparation of FET.
Study design, size, duration: This retrospective cohort study included 164 women with PCOS undergoing FET between Jan 2014 and Dec 2015.
Participants/materials, setting, methods: In letrozole group, patients were administered 5 mg letrozle per day from menstrual cycle day (MCD) 3. By ultrasound examination and monitoring of serum hormone levels, exact ovulation day was assessed. Cryopreserved day 3 embryos were transferred 3 days after ovulation.

In HRT group, patients were administered 6 mg estradiol valerate from MCD 3. When endometrial thickness was above 8 mm, 50 mg intramuscular progesterone was injected. Day 3 embryos were transferred 3 days after progesterone injection.
Main results and the role of chance: In each group, age, AMH level, number of transferred embryos and percentage of top quality embryos reflected no differences. Pregnancy rate of letrozole group showed increased tendency as compared with that of HRT group [55.6%(25/45) vs. 39.5%(47/119), P = 0.094]. There was no significant difference between two groups in the spontaneous abortion rate [12.0%(3/25) vs. 29.7%(14/47), P = 0.283]. Ongoing pregnancy rate was significantly higher in letrozole group [48.9%(22/45) vs. 27.7%(33/119), P = 0.029].
Limitations, reasons for caution: This is a retrospective study. A prospective randomized study with large sample size would have minimized potential limitations.
Wider implications of the findings: FET after ovulation induction, patient-friendly endometrial preparation method without painful injection, showed significantly higher ongoing pregnancy rates than FET after HRT cycle. It suggests not only estrogen and progesterone but also another substance could affect implantation. Study about endometrium after HRT and ovulation induction could help better understanding about implantation.
Trial registration number: None.

P-183 Elective single compared to double blastocyst transfer in women aged 40–43 years of age

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Study question: Does elective single blastocyst transfer (eSBT) compromise the reproductive outcome in women 40–43 years of age?
Summary answer: For older women, eSBT can be applied, with live birth rate comparable to elective double blastocyst transfer (eDBT), but with reduced chance of multiple births.
What is known already: Various studies have shown the effectiveness of elective single embryo transfer in young women in achieving comparable live birth rate, and significantly reducing the rate of multiple births. The role of elective embryo transfer in older women is still controversial with conflicting results, mainly due to scarcity of studies. According to our knowledge elective blastocyst transfer without preimplantation genetic screening (PGS) in women aged at least 40 years has not been studied before
Study design, size, duration: This was a retrospective cohort study that included all women aged 40 years and above, who underwent fresh, non-donor elective blastocyst transfer, where supernumerary blastocysts were available for vitrification, between January 2011 and June 2015. Gonadotropin-releasing hormone (GnRH) agonist downregulation or GnRH antagonist protocols were used. Embryos were cultured to the blastocyst stage and transferred on day 5, while any remaining embryos of good quality (≥3BB) were vitrified on day 5 or 6.

Participants/materials, setting, methods: 221 women were included, where one blastocyst (eSBT, $n = 146$) or 2 blastocysts (eDBT, $n = 75$) were transferred. According to our guidelines, up to 2 blastocysts can be transferred in this age group. The decision regarding the number of transferred blastocysts was dependent on the number of available embryos and opinion of the treating physician and couples. Women with 4 or more previous IVF cycles were excluded. Chi-squared tests and logistic regression controlling for confounders were used.
Main results and the role of chance: The live birth rate in the whole group was 22.62% ($n = 50$). Women with eSBT were significantly younger (40.74 ± 0.84 vs. 41.03 ± 0.82 years, $p = 0.016$) and had significantly fewer previous IVF cycles compared to women with eDBT (0.51 ± 0.8 vs. 1.07 ± 1.02 cycles, $p < 0.0001$). eSBT and eDBT had comparable; peak stimulated serum estradiol levels (7414 pmol/l vs. 8031 pmol/l, $p = 0.26$), number of Metaphase II oocytes collected (9.69 ± 4.06 vs. 10.82 ± 4.04, $p = 0.057$) and numbers of supernumerary vitrified embryos (2.29 ± 1.5 vs. 2.23 ± 1.8, $p = 0.7$), respectively. eSBT and eDBT had similar clinical pregnancy rate (32.87% vs. 42.66%, $p = 0.18$), live birth rate (19.86% vs. 28.0%, $p = 0.17$) and miscarriage rate (34.37% vs. 39.58%, $p = 0.81$,) respectively. There were 5 twin deliveries after eDBT, but there were none after eSBT (23.80% vs. 0%, $p = 0.009$).
Limitations, reasons for caution: The retrospective nature of the study and the small number of women in each group. The decision to transfer more than one embryo was tailored per patient based on clinician and patient discussion and could be a source of bias.
Wider implications of the findings: Reducing the risk of multiple pregnancies is of paramount importance in older women who are at increased risk of adverse perinatal outcomes. This study shows that eSBT achieves comparable live birth rate to eDBT, while reducing the multiple live birth rate. Larger studies are needed to approve these results.
Trial registration number: N/A.

P-184 Effect of vitrification-warming cycle at the blastocyst stage using two types of closed vitrification systems

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Study question: To investigate which is a more valuable closed vitrification system for blastocysts Rapid-i system or control system.
Summary answer: Significantly higher pregnancy rates, along with higher survival rates post-warming, were obtained in the Rapid-i system group compared to the control system group.
What is known already: Embryo cryopreservation is an important technique in assisted reproductive technology (ART). The closed vitrification system is a safety method employed to avoid direct contact between the embryo and liquid nitrogen, and to further avoid possible cross-contamination. Recently, new devices have been developed that have had a faster cooling and warming rate. This study aims to compare embryo viability after exposure to vitrification by using two types of closed systems: the Rapid-i or the control.
Study design, size, duration: During a prospective study period between September 2013 and July 2015, 316 and 306 blastocysts that were vitrified using the Rapid-i and the control system were thawed, respectively. The survival, pregnancy and miscarriage rates were compared.
Participants/materials, setting, methods: The blastocysts were randomly vitrified on days 5/6. After warming, the blastocysts were judged to have "survived" if >40% of the features were intact. During all transfer cycles, hormone replacement treatment (HRT) was administered. A single blastocyst was transferred into the patients' uterus.
Main results and the role of chance: The embryo survival rate from the Rapid-i group was 90.2% (285/316), and 81.7% (250/306) in the control group ($P < 0.01$). The clinical pregnancy rates from embryo transfer cycles in the Rapid-i group and the control group were 39.9% (107/268) and 30.3% (71/234), respectively ($P < 0.05$). The miscarriage rates from embryo transfer cycles were 37.4% (40/107) for the Rapid-i group and 39.4% (28/71) for the control group (not significantly different). Chromosomal anomaly rates derived from the blastocysts that failed to continue to pregnancy were 66.7% (12/18) and 45.5% (5/11), respectively.
Limitations, reasons for caution: Clinical outcomes should be extended to compare live birth rates and birth defect rates between the two groups. Additional long-term follow-up research may demonstrate more evidence about the closed vitrification method's efficacy.
Wider implications of the findings: This study shows that the Rapid-i system for the vitirification of blastocyst has a higher survival and pregnancy rate than the control system. Further studies later in the fetus development are needed to confirm long-term efficacy.
Trial registration number: Not applicable.

P-185 Observation of normal cytokinesis at first mitosis 26 hours after insemination is effective in predicting embryo development

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Study question: Is the normal cytokinesis at 1st mitosis effective in predicting subsequent embryo development 26 hours after insemination?
Summary answer: Embryos that underwent normal cytokinesis at 1st mitosis until 26 hours after insemination had high developmental potential compared with late-cleaved or abnormally-cleaved counterparts.
What is known already: Data of time-lapse cinematography (TLC) has shown that normal cytokinesis at 1st mitosis is one of markers to predict the development to the blastocyst in human ART. In addition, the average time required for the start of 1st mitosis in embryos which developed to blastocysts was 26 hours after insemination. In this study, we examined whether we could predict the subsequent embryonic development by assessing the normal cytokinesis at 1st mitosis 26 hours after insemination using an inverted microscope, not TLC system.
Study design, size, duration: This study included 3165 embryos that underwent normal fertilization after IVF or ICSI between October 2014 and March 2015. The couples received full explanations regarding the study and gave their consent to being involved.
Participants/materials, setting, methods: We divided into two groups according to the cleavage pattern at 1st mitosis 26 hours after insemination (2 cell: Group A vs. 1 cell or abnormally-cleaved embryos: Group B). And then, embryos were cultured individually. Single embryo transfer was conducted on day 3. One morphologically-good embryo was selected for transfer. We investigated the rates of good quality embryo (GQE) on day 3, of blastulation, of good quality blastocyst (GQB) on day 5, and of implantation.
Main results and the role of chance: The normal cytokinesis at 1st mitosis was observed in 779 embryos until 26 hours after insemination (24.6%). The rate of GQE in Group A was significantly higher than that in Group B ($P < 0.0001$, 90.8 vs 62.0%). The rates of blastulation and GQB in Group A (70.7 and 40.4%) were also significantly higher ($P < 0.0001$) than those in Group B (37.8% and 22.4%). After single embryo transfer on day3, the implantation rate in Group A was significantly higher than in Group B ($P < 0.01$, 40.9% vs 17.9%).
Limitations, reasons for caution: This study includes all cases obtained informed consents in the period without considering the patients' characteristics. So, we would like to assess effects of maternal age and stimulation protocol in the future.
Wider implications of the findings: The observation of 1st mitosis 26 hours after insemination with a conventional microscope would be helpful to predict embryo development without using a high-priced TLC system.
Trial registration number: None.

P-186 Introduction of Follicular Fluid Activation ICSI (FFA-ICSI): retrospective investigation of a culture result and a clinical outcomes

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Study question: We performed a retrospective analysis of results derived from a culture result and clinical studies of Follicular Fluid Activation intracytoplasmic sperm injection (FFA-ICSI) and conventional ISCI (N-ICSI).

Summary answer: Compared to N-ICSI, FFA-ICSI showed better results in total fertility rate, normal fertilization rate, blastulation rate, top grade blastocyst rate and pregnancy rate.
What is known already: It was previously reported that addition of follicular fluid had stimulated activation of sperm and contributed to the process of capacitation.
Study design, size, duration: We divided mature eggs retrieved from each patient into two groups, the FFA-ICSI group and the N-ICSI group, and analyzed obtained results in five different categories. 73 cases of ICSI performed in our hospital between January, 2013 and September, 2014 were subjected to this analysis.
Participants/materials, setting, methods: Among the cases where ICSI was applied in our hospital, patients from which we could collect eggs from multiple ova were subjected to this analysis. Cases where female patients had anti-sperm antibodies and endometriosis were excluded from this analysis. Follicular fluid was added in washed sperm 90 minutes prior to ICSI, and ova of each patient were divided into the FFA-ICSI group and the N-ICSI group. We then enforced ICSI.
Main results and the role of chance: The results for the FFA-ICSI group and N-ICSI group, shown in percentage and actual numbers in parenthesis, are as follows: total fertility rate {90.9% (239/263) vs. 86.1% (192/223)}, normal fertilization rate {83.6% (220/263) vs. 76.2% (170/223)}, blastulation rate {64.7% (141/218) vs. 53.4% (93/174)}, top grade blastocyst rate {45.4% (99/218) vs. 37.4% (65/174)}, clinical pregnancy rate{45.0% (86/191) vs. 43.8% (42/96)}. In this analysis, a significant difference was observed in one of the categories. FFA-ICSI showed a better outcome than N-ICSI in other categories. There is a possibility that the Follicular Fluid technique has improved the outcome of ICSI, and contributed to the increase of pregnancy rate.
Limitations, reasons for caution: With regard to pregnancy rates, we transferred embryos with the highest grades. Therefore, there is a possibility that the deviation was observed because of the differences between embryos derived from FFA-ICSI or N-ICSI.
Wider implications of the findings: Increasing number of available embryos by blastulation rate is better, there is a possibility to contribute to the increase pregnancy rate.
Trial registration number: Not applicable.

P-187 Successful blastocyst biopsy and Preimplantation Genetic Screening after embryo cryopreservation and extended culture: analysis on 213 frozen-thawed supernumerary embryos, previously cryopreserved without biopsy

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Study question: To evaluate the efficacy of performing extended culture until blastocyst stage, trophectoderm biopsy, whole genome amplification and preimplantation genetic screening (PGS) on previously cryopreserved embryos.
Summary answer: Performing PGS at blastocyst stage on cleavage embryos previously cryopreserved without biopsy and subsequent genetic analysis, allows to achieve excellent clinical and biological outcomes.
What is known already: In the last 10 years, the enhancement of cleavage stage embryo's and blastocyst's vitrification procedures, led to very good survival rate and clinical outcomes. Simultaneously, the improvement in blastocyst biopsy combined with the analysis of all the 24 chromosomes in PGS cycles, allowed to achieve pregnancy rates per transfer higher than 60%. Combining these two methodologies could offer concrete possibility of delivery a healthy baby to all couples who still have their supernumerary embryos cryopreserved in the past years.
Study design, size, duration: This retrospective study was performed from January 2011 to December 2015. Fifty-one couples required to be informed about the genetic constitution of their own supernumerary embryos, previously cryopreserved without any genetic screening. All embryos were vitrified at cleavage stage. All survived embryos were cultured until blastocyst stage at 37°C, 6%CO^2, 5%O^2, in order to perform trophectoderm biopsy. All biopsies were analyzed by mean of whole genome amplification (WGA) and array-comparative genomic hybridization (aCGH).
Participants/materials, setting, methods: The mean female age was 36.3 ± 4.23. Only euploid blastocysts were transferred. Depending on when the blastocysts were obtained, some transfers were performed the day after the biopsy, as soon as receiving the genetic results (group-A), while the other blastocysts were once again individually cryopreserved, in order to perform the transfer in a subsequent cycle (group-B). In group-A, 5 double and 24 single embryo transfers were performed. In group-B, 15 single embryo transfers were performed.
Main results and the role of chance: A total of 217 cleavage stage embryos were warmed and 213 (98.2%) of them survived. After the extended culture, 146 (68.5%) or them reached the blastocyst stage, 97 (66.4%) on day-5, 43 (29.5%) on day-6 and 6 (4.1%) on day-7 of culture. After the genetic analysis, 62 (42.5%) of them resulted euploid, 8 (5.5%) mosaic, 71 (48.6%) aneuploid and 5 (3.4%) without genetic result. Forty-nine euplodid blastocysts were transferred in 44 transfers, leading to cumulative clinical pregnancy (CPR) and implantation (IR) rates of 61.4% (N = 27) and 55.1% (N = 27), respectively. In group-A, 34 blastocysts were transferred in 29 transfers, leading to CPR of 55.1% (N = 16) and IR of 47.1% (N = 16); 14 healthy babies born, 1 pregnancy is still ongoing and 1 miscarried. In group-B, 15 blastocysts were transferred in 15 single embryo transfer transfers, leading to CPR and IR of 73.3% (N = 11); 3 healthy babies born, 4 pregnancies are still ongoing and 4 miscarried. There are no statistically significant differences in clinical outcomes between group-A and group-B. Statistical analyses were performed using the Fisher's exact test at the level of $P < 0.05$.
Limitations, reasons for caution: Performing double vitrification, some blastocyst could be lost due to the cryopreservation procedures. Performing the transfer on the day after the biopsy after receiving the genetic result, the endometrial receptivity could be altered.
Wider implications of the findings: Embryos cryopreserved before the advancement of the PGS procedures, can now be tested before proceeding with their embryo transfer, in order to improve the clinical outcomes obtaining a safe pregnancy. Performing the biopsy at blastocyst stage do not interfere with embryo's development, probably because only few extra-embryonic cells are removed.
Trial registration number: Not applicable.

P-188 Expression of Glucose Metabolism Genes in Human Embryos Cultured in a Sequential Media System by Single-cell RNA-seq

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Study question: Using single-cell RNA-seq and Weighted Gene Co-expression Network Analysis (WGCNA) to explicit expression of glucose metabolism genes in human pre-implantation embryos cultured in the sequential media.
Summary answer: Glucolysis genes are gradually increase and highly upregulated in morula and blastocysts compared to cleavage-stage embryos, and tricarboxylic-acid-cycle genes are fluctuant through all developmental stages.
What is known already: Different stage embryos require different glucose metabolism. Zygotic genome activation (ZGA) is a gradual process or a stringently controlled program, and glucose metabolism is regulated by ZGA with specially appointed features in different stages of pre-implantationembryos.
Study design, size, duration: Human pre-implantation embryos were obtained from patients who underwent IVF treatment and written informed consent was obtained. Total of 39 oocytes and embryos were used in this study: 6 oocytes, 6 pronuclei, 4 zygotes, 5 2-cellstage embryos, 4-cellstage embryos, 5 8-cellstage embryos, 4 morula and 4 blastocysts. After digestion, 78 cells or blastmeres were used for construction of single-cell RNA-library.
Participants/materials, setting, methods: Human pre-implantation embryos were cultured in the sequential media (SAGE, USA) including cleavage- and blastocyst-media. Embryosat different stages were treated with Tyrode's acidic solution to remove zona pellucida, and then be separated by 0.25% Typsin digestion to acquire single cells that were used to construct RNA library for RNA-seq data using Quartz-seq and SMART-seq methods. After raw reads being mapped to the human genome, all datasets were independently constructed and analysis using the WGCNA.

Main results and the role of chance: The expressions of glucolysis genes (relationship to anaerobic respiration) are speedily increasing in morula and reach the highest in blastocysts, e.g., glucosetransporters (SLC2A1, SLC2A3 and SLC2A8) were not expressed before 8-cell stage until morula. Moreover, three key genes of glucose catabolism (hexokinase, phosphofructokinase and pyruvate kinase) could not be detected in 2-, 4- and 8-cell stage embryos but in morula and blastocysts. However, expressions of tricarboxylic acid cycle genes (relationship to aerobic respiration) were fluctuant, e.g., pyruvate dehydrogenase (PDHB/DLD) and citrate synthase (CS), key enzymes of tricarboxylic acid cycle, were slightly high-expressed in 2-cell stage and morula but were lowly expressed in 8-cell stage embryos.
Limitations, reasons for caution: In this study, every stage of human pre-implantation embryos was not cultured in gradient concentration glucose media to detect effects of concentrations on glucose metabolism in the embryos.
Wider implications of the findings: The results provide a valuable information to improve the composition of culture medium for human pre-implantation embryos. Also this study provides new insight into sequential activation of metabolism-specific genetic programs and gene regulatory mechanisms underlying progressive development of early human embryos.
Trial registration number: None.

P-189 Diameter of immature oocytes collected in IVM cycles and its association with nuclear maturation timing

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Study question: Study questions: What is the *in vitro* maturation timing of human Germinal Vesicle (GV)-stage oocytes collected from IVM cycles? Is oocyte diameter at GV-stage associated with meiotic competence?
Summary answer: Summary answer: The GV-period is shorter than Germinal Vesicle Breakdown (GVBD)-stage *in vitro*. Oocyte diameter at GV-stages is an indicator of oocyte maturation capacity
What is known already: IVM has been less successful than standard IVF in terms of embryo development and clinical outcomes. The nuclear oocyte maturation timing process of immature GV-stage oocytes during *in vitro* culture hasn't been known. Previous studies have linked oocyte size to the maturation status but no direct correlation has been made. It is currently unclear whether the diameter is different between IVM oocytes and *in vivo* matured oocytes derived either from same cohort in IVM cycles or from conventional IVF cycles.
Study design, size, duration: The maturation timing process from GV-stage oocytes obtained was recorded following culture by adopting the EmbryoScope time-lapse system (TLS) equipment. Seventy-five MII- and 193 GV-stage oocytes were possible to analyze under TLS. The average size of oocytes were compared in oocytes matured *in vitro* ($n = 93$), arrested at GV-stage ($n = 100$) and matured *in vivo* of same cohort ($n = 75$) using the ellipse function of the TLS.
Participants/materials, setting, methods: The oocytes were obtained from PCOS patients undergoing FSH/hCG-primed IVM cycles. Most of the cumulus cells (CC) were removed 3 hours after oocyte retrieval in order to see the oocyte maturation. GV-stage oocytes were cultured for 28 hours in the TLS prior to ICSI. The diameters of the GV-stage oocytes and maturation timing were recorded. All oocytes matured or arrested after 28 hours of culture were included in this study.
Main results and the role of chance: The average time from GV- to GVBD-stage was 6.3 hours (±2.3, range: 3.5–12.3 hours) after retrieval and GBVD- to MII-stage was 15. 5 hours (±1.5, range: 10.7–18.6 hours) during culture *in vitro*. Analysis of oocytes at the GV-stage revealed that oocytes matured *in vitro* ($n = 93$) had significantly larger diameters (μm) at GV-stage than those that failed to resume meiosis after IVM ($n = 100$) (110.7 ± 4.8 versus 106.7 ± 4.6 μm; $P < 0.0001$). Interestingly, no GV-stage oocyte with average diameter < 102 μm matured *in vitro* after 28 hours of culture ($n = 14$). The diameter did not change from GV- to GVBD-stage, but decreased after the first polar body extrusion (109.1 ± 4.9 μm). The average diameter at the MII-stage matured *in vitro* were similar with cohort of *in vivo* matured oocytes in IVM cycles (108.8 ± 3.3 μm) and oocytes matured *in vivo* obtained from conventional IVF cycles (108.6 ± 3.6 μm).
Limitations, reasons for caution: Most of CC at GV-stages were removed, which may act differently than oocytes with intact CC. Maturation timing may be different between different sources of immature oocytes. Studies are required to clarify the maturation timing of immature oocytes depending on CC presence, culture media and different sources of immature oocytes.
Wider implications of the findings: This is the first observation describing thoroughly the *in vitro* maturation process of GV-stage oocytes derived from IVM cycles using TLM. This study clearly demonstrated a direct correlation between the diameter at GV-stage and oocyte maturation capacity *in vitro*. This study may provide essential information for improving human IVM program.
Trial registration number: Not applicable.

P-190 Follicle size and synchronicity of follicular development influence morphokinetic variables in embryos

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Study question: Are morphokinetic parameters of embryo development influenced by follicular size (large and small) in synchronous and asynchronous follicle development during stimulation?
Summary answer: Embryos from small follicles in asynchronous cycles develop significantly faster starting from 5-cell stage up to expanded blastocyst stage.
What is known already: Several studies have investigated the effect of culture media, culture conditions, patients' etiology, smoking habit, BMI and stimulation types on embryo morphokinetics. These parameters were reflected to different extents in subtle changes in morphokinetic variables. However, whether similar changes occur in implanting embryos or if these follow a more universal pattern, is currently unknown. Since the implementation of time-lapse systems, clinic-specific morphokinetic models have been proposed. In search of a general applicability of morphokinetic models, potential confounding factors that can be objectively assessed need to be investigated. This holds true for synchronicity of follicular development and size.
Study design, size, duration: This retrospective cohort study was conducted in a private IVF clinic between July 2014 and September 2015. Strict inclusion criteria (<2 previous treatment cycles, age ≤ 39 years, ≥8 oocytes retrieved) and exclusion criteria (PGD or PGS indication, > 24 COCs during pickup) were applied. Morphokinetic analyses were performed only for fertilized oocytes achieving the blastocyst stage ($n = 1217$) derived from 187 infertile patients.
Participants/materials, setting, methods: Synchronous cycles were defined as follicles of all sizes being present, whereas asynchronous cycles were those clearly separated into a small and a large cohort. Small follicles were defined as <17 mm on OPU day. Culture was performed in EmbryoScope at 6%CO2 and 5%O2 using a single-step culture medium (Global) with change on day3. Morphokinetic variables for all cleavage events up to the expanded blastocyst stage were annotated. Embryo selection was done according to morphology.
Main results and the role of chance: Embryos developing from small follicles in asynchronous cycles were found to achieve all cleavage times earlier than those developing from large follicles in asynchronous cycles but also than small and large follicles developing in synchronous cycles. At the end of the culture, the time to reach the expanded blastocyst stage (tEB) was 3 hours earlier in small follicles of asynchronous cycles when compared to the other three categories.

Next we looked at the timings of embryos with known implantation data (KID). For implanting blastocysts (KID positive) the mean time for tEB was 110 h for those developed from small and large follicles in synchronous cycles and from small follicles in asynchronous cycles, whereas implanting blastocysts from large asynchronous follicles showed a different mean tEB time (tEB 113 = h).
Limitations, reasons for caution: Inclusion and exclusion criteria favored a group of good prognosis patients. Results were obtained in a defined IVF setting and cannot necessarily be translated in another laboratory.
Wider implications of the findings: Constructing optimized morphokinetic algorithms for a specific center needs to take into account further variables such as follicular size and synchronicity. This is of uttermost importance if the final goal is to use models for identifying embryos for an elective single embryo transfer.
Trial registration number: Not applicable.

P-191 Proliferation of two sources of trophectoderm cells from the same cohort in human blastocysts. A time lapse imaging report

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Study question: Is it possible to transplant and proliferate trophectoderm cells in blastocysts from the same cohort with low number of trophectoderm cells?
Summary answer: A successful transplant and proliferation of trophectoderm cells were achieved in blastocysts with low number of trophectoderm cells.
What is known already: Approximately 80% of human blastocysts are discarded because they do not reach the optimal quality to provide a chance of a clinical pregnancy and/or a birth outcome. Blastocyst quality is expressed in terms of the number of trophectoderm cells as well as the inner cell mass during this stage of embryo development. To be successful, a few embryos must be of good quality in the trophectoderm area. Trophectoderm morphology has been considered a highly independent predictor of both clinical pregnancy and birth outcome. Blastocysts with a higher number of trophectoderm cells correlate significantly with a higher implantation rate.
Study design, size, duration: Embryos that were non-viable that would subsequently be discarded were selected for this study based on the quality of the outer cells. Specimens were derived from unused cells obtained from *in vitro* fertilization patients who had consented to have these discarded cells used for research purposes. Embryos were monitored using a time lapse imaging system (EmbryoScope™, Vitrolife).
Participants/materials, setting, methods: Approximately 8–10 trophectoderm cells were injected into two blastocysts from the same cohort with a low number of trophectoderm cells. An assisted 30 μm hole in the zona pellucida was needed to inject the trophectoderm cells. Blastocysts were placed in the embryoScope and cultured with G2 plus media (Vitrolife) for 20 hours. Images were acquired every 20 minutes from 7 different focal planes. Cellular activity was monitored every 20 minutes.
Main results and the role of chance: A successful transplant and proliferation of trophectoderm cells were achieved in two blastocysts with low number of trophectoderm cells. Cellular activity started within the first 20 minutes with an increment and proliferation of trophectoderm cells from 113 μm on diameter and an area of 11031 μm^2. Blastocysts started hatching approximately 40 minutes after cell transplantation with and increment of 35% of blastocyst perimeter and 40% of blastocyst area (172 μm and 18309 μm^2, respectively). Cell growth continued until a blastocyst hatched stage. Hatched blastocysts were removed from the EmbryoScope and placed in a 15 microliter drop for a final video evaluation.
Limitations, reasons for caution: These results are the preliminary findings of a research project involving the transplant and proliferation of trophectoderm cells in human blastocysts. More subjects are needed to understand the attachment and proliferation of trophectoderm cells from two different sources.
Wider implications of the findings: The intended result is the rescue of more embryos that may be considered viable for a fresh transfer and/or cryopreservation with a higher rate of clinical pregnancy and/or birth. If successful, this novel technique may result in a 10–15% increase in the potential for pregnancy.
Trial registration number: USA Institutional Review Board (IRB) # 201505154 Protocol #: SAIRB-15-0062.

P-192 Impact of exposure time of fresh and frozen-thawed blastocysts to Hyaluronic acid containing transfer medium

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Study question: Is the EmbryoGlue exposure time prior to transfer in fresh and frozen-thawed (FET) single blastocyst transfers (SBT) associated with the probability of clinical pregnancy?
Summary answer: No correlation between EmbryoGlue exposure time and clinical pregnancy rates was detected for both fresh and frozen-thawed SBT.
What is known already: A recent Cochrane systematic review (Bontekoe et al., 2014) has shown that the use of Hyaluronic Acid (HA) in embryo transfer media is associated with higher live birth and clinical pregnancy rates in in-vitro fertilization cycles when compared to the use of low or minimal HA. The recommended exposure time for embryos to EmbryoGlue prior to transfer has traditionally been short (10–30 min). However, it is still unknown whether a longer duration of embryo exposure to HA before transfer is associated with the probability of pregnancy.
Study design, size, duration: This is a retrospective cohort analysis of 931 consecutive cases of fresh ($n = 489$) and frozen-thawed ($n = 442$) SBT that took place over a period of ten weeks.
Participants/materials, setting, methods: Patient age ranged from 21.2 to 52.1 years (mean: 36.5 years and SD: 4.7) with EmbryoGlue exposure times ranging from 7 to 720 min (mean:115 min, SD:82.9). Four groups were constructed (Group 1: ≤60 min, Group 2: 61–120 min, Group 3: 121–180 min, Group 3: >180 min). The correlation between exposure time prior to transfer with clinical pregnancy rate was evaluated using logistic regression analysis, while controlling for the confounding effect of female age.
Main results and the role of chance: In fresh SBT, longer exposure to EmbryoGlue did not result in significantly different clinical pregnancy rates as compared to ≤60 min (Group 2 vs. Group 1 odds ratio (OR): 0.61, 95% CI: 0.36–1.03; Group 3 vs. Group 1 OR: 0.65, 95% CI: 0.36–1.18; Group 4 vs. Group 1 OR:0.75, 95% CI: 0.41–1.36). Similarly, in frozen-thawed SBT, longer exposure to EmbryoGlue also did not result in significantly different clinical pregnancy rates as compared to ≤60 min (Group 2 vs. Group 1 OR: 1.02, 95% CI: 0.62–1.66; Group 3 vs. Group 1 OR: 1.67, 95% CI: 0.91–3.05; Group 4 vs. Group 1 OR:1.22, 95% CI: 0.67–2.22).
Limitations, reasons for caution: This is a retrospective analysis and although adjustment for major confounding has been performed, the presence of residual bias cannot be excluded.
Wider implications of the findings: These results do not support either a positive or negative effect of prolonged EmbryoGlue exposure on clinical pregnancy rates after Fresh or Frozen-thawed transfer. Embryologists can organize their embryo transfers without being concerned about the duration of embryo exposure to EmbryoGlue.
Trial registration number: Nil.

P-193 Comparative assessment of human blastocyst resiliency to vitrification solution toxicity and osmotic stress associated with re-vitrification (rVTF)

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Study question: What is the cryotolerance of human blastocysts to repeated re-vitrification (rVTF) and varying exposures to different VTF solutions as sources of osmotic injury and cytotoxicity?
Summary answer: Human blastocysts are more resilient to extended vitrification solutions exposures and to the potential osmotic stress of repeated rVTF (≤6 times) than previously assumed.
What is known already: Vitrification has proven to be a highly efficient mode of embryo cryopreservation. Vitrified embryo survivability is dependent on: 1) exposure to concentrated cryoprotective solutions that form a glasseous solid upon rapid cooling; and 2) high warming rates followed by cellular elution to an isotonic, equilibrated condition. We have previously shown that human embryos are highly adaptive to osmotic shifts during elution and that the rVTF of aneuploidy blastocysts can serve as a useful model. Current dogma suggests that high molar concentrated vitrification solutions (>5.5 M) exposures must be brief to avoid cellular toxicity and that embryos are susceptible to osmotic stress.
Study design, size, duration: 360 research consented, discard blastocysts were randomly assigned to: Expt.1) 2 × 3 factorial design: repeated rVTF (1, 3 or 5 times) with or without elution/equilibration; or Expt.2) 6 × 3 factorial design: comparing 6 exposure intervals post-warming (1, 3, 5, 10, 15, 20 min/20 blastocysts/group) in a commercial EG/DMSO (15/15% solution; LifeGlobal), EG/PPG (16/16% solution; Vitrolife) or our control Glycerol/EG (≥7.9 M; Innovative Cryo Enterprises) vitrification solutions. Differences in % survival and %24 hr development were statistically compared by Chi-square analysis.
Participants/materials, setting, methods: Biopsied blastocysts (AA to BB quality) were vitrified using microSecure vitrification (μS-VTF), an aseptic closed system. Upon determination of their aneuploidy status, embryos were discarded or assigned a research treatment and discarded within 24 hr. Standard μS-VTF rapid warming was performed on all embryos, with non-elution treatment blastocysts (Expt.1) simply being wiped dry and reinserted/sealed

into straws and plunged back into LN_2 1-min post-warming. Embryo survival assessments were performed at 0 and 24 hr (i.e., continued expansion) post-treatment.

Main results and the role of chance: Human blastocysts proved to be highly cryotolerate to extended VTF solution exposure (with or w/o VTF), dilutions/elutions and repeated rVTF (up to 5 times), with no differences in post-warming or developmental survival (95–100% expansion) being observed in Expt.1. However, in Expt.2, 24 hr development was significantly reduced with ≤5 min exposure in the EG/DMSO group (83.3%), compared to EG/PPG (93.3%) or Glycerol/EG (96.7%). Interestingly, the developmental integrity of Glycerol/EG treated blastocysts reduced (75%; $p < 0.05$) by 10 min and was significantly lower at 15/20 min (30–35%) than either EG/DMSO (55–70%) or EG/PPG (55–60%). In Expt.1, the high survival rate of non-eluted rVTF-treated blastocysts was facilitated by repeated rapid warming. Conversely, the elution group endured repeated osmotic stress characterized by cellular shrinkage and re-expansion in sucrose and vitrification solutions. The plasticity and cryotolerance of blastocyst to vitrification treatment was dramatically demonstrated on our control group (µS-VTF in Glycerol/EG), which proved to be very safe up to 5 min. Note, that the Glycerol/EG group has an appreciably higher solute concentration than the other solutions, insuring more metastability in its vitrified "glassy" state without the ultra-rapid cooling of an open-system. However, the latter solution was more toxic with prolonged cryoprotectant exposures >10 min.

Limitations, reasons for caution: The genotoxicity of all treatment solutions remains unknown, especially in the extended exposure groups (>5 min). Certainly 2 min exposures and 2X rVTF are regarded as safe, having previously yielded proven live births. Additional investigation is needed to determine how EG/DMSO –treated blastocysts will respond to repeated rVTF.

Wider implications of the findings: Human blastocysts are highly resilient to rVTF, with or without repeated elutions, and extended exposures to non-DMSO containing VTF solutions. The safety and efficacy of VTF was clearly shown to be highly tolerable by the functional cell membrane integrity sustained after repeated metastable rVTF.

Trial registration number: None.

P-194 Day 2 to 4 embryo transfers, with timing of syngamy as an additional selection criterion, produces similar clinical outcomes as day 5 transfers

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Study question: Does further selection of embryos based on the timing of syngamy for day 2 to 4 transfers achieve clinical outcomes comparable to day 5 transfers?

Summary answer: Incorporating timing of syngamy with development and morphology for embryo selection allows day 2 to 4 transfers to achieve clinical outcomes of day 5 transfers.

What is known already: Culturing embryos to day 5 for transfer serves as a selection for embryos that have a higher chance of implantation. Day 5 transfers are proven to give better clinical outcomes than day 2 to 4 transfers. The absence of pronuclei in an embryo 26 hours after intracytoplasmic sperm injection or sperm insemination indicates that it has reached its syngamy stage. This implies that it is a more developmental competent embryo.

Study design, size, duration: This retrospective study includes 264 patients who underwent IVF cycles between August 2013 and December 2015 and had 2 embryos for embryo transfer. The patients were categorized into two groups. Group A consisted of patients with both embryos that had reached syngamy (at 26 hours after injection or insemination) for transfer, either on day 2, 3 or 4. Group B consisted of patients with day 5 embryos for transfer, regardless of their timing of syngamy.

Participants/materials, setting, methods: Selection of embryos for transfer was based on embryo development and morphology on the day of embryo transfer in both groups. All the patients were aged between 27 and 45 years old. Group A had 126 patients (average age: 35.18) and Group B had 138 patients (average age: 35.84). The outcomes compared were clinical pregnancy, implantation and live birth rates.

Main results and the role of chance: Analysis of the results through chi-square test proved that there were insignificant differences between the two groups in terms of clinical pregnancy, implantation and live birth rates. In Group A, the clinical pregnancy rate was 44.4% which is not significantly different from the 42.0% of Group B (p-value = 0.69). Implantation rates were 29.4% and 31.5% for Group A and B respectively and the difference was negligible (p-value = 0.59). This is also the same for live birth rate (p-value = 0.43), where Group A has the rate of 77.2% with 15 ongoing pregnancies while Group B has 70.7% with 19 ongoing pregnancies. These results suggest that embryos selected for embryo transfer from day 2 to 4, based on timing of syngamy together with embryo development and morphology, possessed the implantation potential of embryos selected for Day 5 transfers.

Limitations, reasons for caution: Only fresh IVF cycles were included in this study. The addition of frozen embryo transfer data may affect the cumulative pregnancy rate of the two groups.

Wider implications of the findings: By considering timing of syngamy, day 2 to 4 transfers can achieve comparable clinical outcomes to day 5 transfers. This provides a possible option when resources to perform blastocyst culture are limited. It will encourage single embryo transfer practice and reduce potential risks associated with multiple pregnancies and extended culture.

Trial registration number: NA.

P-195 Aberrant cell division in early-stage human embryos observed by time-lapse monitoring and its negative impacts on subsequent embryonic development

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Study question: Does aberrant cell division at an early stage of human embryonic development have negative impacts on further embryonic development?

Summary answer: Aberrant division was associated with retardation of embryonic development and an increased incidence of multinucleated blastomeres (MNB) resulting in poor-quality embryos.

What is known already: In recent years, incubators equipped with time-lapse monitoring systems have enabled assessment of the dynamic morphology of human embryonic development. Using such monitoring, we often observe embryos showing aberrant cell division in which a single zygote divides into more than three blastomere-like cells in the first cleavage and a single blastomere into three or more blastomere-like cells in the second cleavage. It seems to be an abnormal behavior of embryos; however, the developmental potential and negative impacts on embryonic quality of embryos showing aberrant cell division have not been elucidated.

Study design, size, duration: From August 2012 to December 2014, intracytoplasmic sperm injection (ICSI) was performed on 2,274 oocytes, which were then cultured for three days in incubators with time-lapse monitoring. We analyzed the occurrence of aberrant division in whole normally fertilized zygotes (n = 1,818), comparing embryos from younger patients (31 to 33-year-olds; n = 372) with an advanced-aged group (41 to 43-year-olds; n = 249). We transferred aberrantly divided embryos reaching fair quality if the only alternative.

Participants/materials, setting, methods: We performed ICSI on matured oocytes 4 hours post-oocyte retrieval. Digital images were acquired at 15-minute intervals for two days with the EmbryoScope®. We evaluated cell-cleavage patterns based on time-lapse images of the first and the second cleavage of blastomeres. We classified a cell division yielding more than three blastomere-like cells (diameter >30µm) from one blastomere as an aberrant division. We also analyzed the time course of embryonic development and clinical outcomes for transferred embryos.

Main results and the role of chance: Of 1,818 zygotes, 246 (13.5%) showed aberrant division, and the occurrence rate was not significantly different between young and advanced-aged groups {12.4% (46/372) vs. 10.8% (27/249)}. The rate of MNB was significantly higher in aberrantly divided embryos (aberrant group) than normally divided embryos (normal group) (63.8% vs. 21.2%, $P < 0.01$). Among embryos in the aberrant group, 63.8% (157/246) and 36.2% (89/246) of embryos showed aberrant division in the first cleavage (aberrant^{1st}) and in the second cleavage (aberrant^{2nd}), respectively. Of 246 embryos, 13 embryos (5.3%) showed aberrant division in both first and second cleavages (aberrant$^{1st/2nd}$).

Time required from ICSI procedure to syngamy in the aberrant group was significantly longer than in the normal group (aberrant^{1st}; 24.5 ± 4.4, aberrant^{2nd}; 24.6 ± 3.1, aberrant$^{1st/2nd}$; 26.8 ± 6.6, and normal group; 23.0 ± 3.6 hours).

Additionally, time required from syngamy to the first cleavage in aberrant[1st] embryos (aberrant[1st] vs. normal; 4.7 ± 3.6 vs. 2.8 ± 1.4 hours, $P < 0.01$) and the first cleavage to the second cleavage in aberrant[2nd] embryos (aberrant[2nd] vs. normal; 13.0 ± 3.6 vs. 11.3 ± 1.7 hours, $P < 0.01$) was significantly longer than in the normal group.

In cases of transferring aberrantly divided embryos, the pregnancy rate was 27% (4/15); three gave healthy babies, and pregnancy is ongoing in the remainder.

Limitations, reasons for caution: It was impossible to determine all nuclei of blastomere-like cells because of focusing difficulties. Aberrant division could be further classified based on whether one blastomere divides into three blastomeres or into two blastomeres and a large fragment. Molecular biological approaches are thus necessary for detecting all nuclei.

Wider implications of the findings: Our study suggested that aberrant cell division was associated with the formation of MNB and a delay in developmental events until the second cleavage. We hypothesized that aberrant cell division could be related to dysregulation of the spindle, controlling the distribution of cytoplasm and chromosomes.

Trial registration number: None.

P-196 An embryo score established with a Time Lapse incubation system to early predict the chance to obtain a blastocyst

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Study question: The objective of this to study is to establish a score based on Day 3 stage morphokinetic parameters obtained with an Embryoscop© **to predict** the chance to obtain a blastocyst.

Summary answer: With few morphokinetics parameters it is possible to obtain a simple score allowing predicting the chance to obtain blastocyst.

What is known already: Blastocyst transfer allows increasing the pregnancy rate. By this way an elective single embryo transfer (eSET) could be proceed in order to minimize the multiple pregnancies. By choosing the embryo at day 3 it could be possible to increase the turnover of embryos and the number of couple benefit the time lapse procedure.

Study design, size, duration: The score was established with a retrospective register cohort study included 564 embryos. The ART procedures were performed from May 2013 to April 2015. In order to avoid oocytes alteration, the exclusions female factors were anti-mullerienne hormon (AMH) less than 0.4 ng/mL; a BMI higher than 35; and less than 5 retrieved oocytes. For men, were excluded ART process with sperm obtained surgically or with frozen. sperm.

Participants/materials, setting, methods: 108 ART cycles were performed. All patients had extended culture to blastocyst using Embryoscop©. The first step was to select the more predictive parameters to predict the blastocyst obtaining. The second step was to establish the score with the constraint of the Embryoscop© software. The Chaid procedure was used to find a cut off value to each selected morphokinetiks parameters. The weight for each class was the coefficient obtained with a Multiple Composante Analysis.

Main results and the role of chance: Among twelve morphokinetics parameters, fours were selected: fading of pronuclei (PNf), t2, S2 and S3. The score range is from 0 to 18. When the score is below to 5, the chance to obtain a blastocyst is equal to 71.6% (78/109), this chance is equal to 83.3% (170/204) when the score is between 5 and 11, and equal to 94.0% (110/117) when the score is higher than 11 ($p < 0.001$). No relationship was found between this score and the female age. A relationship was found between the quality of the blastocyst and the score, the risk to obtain a blastocyst grade C is equal to 35.9% (28/78) when then the score is below to 5 and equal to 20.9% (23/110) when the score is greater than 11 ($p < 0.05$). A relationship was found between the score and the blastocyst stage chronology. When the score is below to 5, a blastocyst at day 5 was obtained in 69.7% (76/109) of cases and in 82.1% (96/117) when the score is higher than 11 ($p < 0.05$). As a eSET was performed for 56 cycles, no relationship has been established between score and pregnancy rate because the size was not sufficient.

Limitations, reasons for caution: A retrospective study was performed to establish the score. This score has to be validated with a prospective study. The study between the score and the pregnancy rate could be studied when eSET is performed.

Wider implications of the findings: With this score, the choice of the transferred embryo could be performed at day 3. A prospective study has to be performed to validate this result. This score should be included in a wide score which included male and female parameters in order to have a global ART procedure score.

Trial registration number: None.

P-197 clinical outcome of frozen embryo transfer versus fresh embryo transfer of 3129 cycles

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Study question: Does freeze all embryos and subsequent frozen embryo transfer (FET) results in better outcomes compared with fresh transfer?

Summary answer: Freezing all embryos and differing transfer to a subsequent endometrial preparation cycle does not improve cycle outcome when compared to fresh embryo transfer.

What is known already: Several studies suggest that endometrial receptivity could be diminished during controlled ovarian stimulation (COS). Some authors have suggested the solution by freezing all embryo and transferring the embryo in a delayed cycle.

Study design, size, duration: It is a retrospective observational cohort study. We include 3129 cycles aged <35 undergoing their first or second IVF/ICSI cycle between January 1st 2013 and December 31st, 2013, in which day 3 embryo transfer was performed.

Participants/materials, setting, methods: The study was performed in the Chongqing reproductive and genetic institute, China. Included 3129 cycles of which 2397 (76.6%) were fresh embryo transfer and 732 (23.4%) were FET. The outcomes of interest were ongoing pregnancy rate, clinical pregnancy rate, implantation rate and take home baby rate.

Main results and the role of chance: No differences were observed between both groups in terms of mean ages and embryo transfer number. The raw analysis showed that clinical pregnancy rate (67.7 vs.68.4%, $p = 1.25$),implantation rate (51.0 vs.48.1%, $p = 3.73$), ongoing pregnancy (62.0 vs.59.6%, $p = 1.40$) and take home baby (59.9 vs. 58.2%, $p = 0.68$) rates for fresh vs. freeze all cycles respectively were similar.

Limitations, reasons for caution: The main limitation is that this is a retrospective study. Furthermore, the cycles number of frozen embryo transfer was small.

Wider implications of the findings: These findings suggest that freeze all embryo could get similar clinical outcomes if patients were not suitable for fresh embryo transfer. But these findings do not support freeze-all strategy in all patients, since the treatment cost and the time to pregnancy is shorter when fresh embryos are transferred.

Trial registration number: NA.

P-198 The correlation between the time interval ICSI performance and hCG administration

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Study question: To assess the correlation between the time interval of hCG administration and ICSI performance for clinical pregnancy outcome intracytoplasmic sperm injection outcome.

Summary answer: There was a strong correlation in the time of ICSI performance following hCG injection for the subsequent clinical pregnancy outcomes.

What is known already: It is well known that oocytes retain their ability to become fertilized much longer than their capacity to develop into viable embryos. Incubation of oocytes for 2–6 h prior to IVF improves fertilization and pregnancy rates. However, the optimal ICSI timing following egg retrieval is still unclear and the existing results are not fully conclusive.

Study design, size, duration: Data were collected from ICSI cycles with day-3 embryo transfers recorded in the Clinical Reproductive Medicine Management System (CCRM) database of our IVF center between January 2010 to August 2015.

Participants/materials, setting, methods: A total of 5,215 ICSI cycles in which oocyte retrieval was performed 36 or more than 36 h after hCG administration were analyzed. According to the length of time interval between ICSI performance and hCG administration, there were six groups, such as: 36–37 h (including 37 h), 37–38 h (including 38 h), 38–39 h (including 39 h), 39–40 h (including 40 h), 40–41 h (including 41 h), >41 h.
Main results and the role of chance: When ICSI was performed in 36–37 hours after hCG administration, the rate of MII oocytes was lower compared to other Groups ($P < 0.05$). The normal fertilization rate had a trend of increasing with time prolonging, and it was significantly lower when ICSI was performed between 36–39 h after hCG administration than performed 39 h or more ($P < 0.05$). The high-score embryo rate had a decreased trend with time prolonging, and it was significantly higher when ICSI was performed between 38–39 h after hCG administration than performed 41 h or more ($P < 0.05$). There was no differences of implantation rate, clinical pregnancy rate and ongoing clinical pregnancy rate among six groups.
Limitations, reasons for caution: The timing of ICSI after hCG administration affected in ICSI outcome, but how to balance the relationship between normal fertilization rate and clinical pregnancy rate needs to be confirmed.
Wider implications of the findings: The timing selection might be important not only for ICSI but also for other timed assisted reproduction techniques.
Trial registration number: –

P-199 IMSI (Intracytoplasmic Morphologically Selected-sperm Injection) versus conventional IVF (*In Vitro* Fertilization): a randomized prospective study about the management of the idiopathic infertility

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Study question: Comparison IMSI *versus* IVF in the management of unexplained infertility of the couple after at least 2 failures of IUI (IntraUterine Insemination) or 1 miscarriage.
Summary answer: Better results in terms of embryo quality and clinical pregnancy and live-birth rates were observed after IMSI.
What is known already: It has been widely reported in randomized studies that IMSI performed after implantation failure(s) after ICSI improved implantation, clinical pregnancy, miscarriage and live-birth rates (Bartoov et al., 2003; Berkovitz et al., 2007; Antinori et al., 2008; Gonzalez-Ortega et al., 2010; Klement et al., 2013), and also the embryo quality (Bartoov et al., 2003; Wilding et al., 2011). It seems also that IMSI is better than ICSI in terms of clinical pregnancy rate and live-birth rate in attempts with sperm high DNA (DeoxyriboNucleic Acid) fragmentation rate (Hammoud et al., 2013).
Study design, size, duration: Prospective cohort study at Bichat-Claude Bernard Hospital (AP-HP, Paris) including 35 couples with unexplained primary or secondary infertility and without pregnancy after at least 2 IUI or 1 miscarriage, between January 2010 and June 2015.
Participants/materials, setting, methods: All couples gave their informed consent. Only couples with normal semen parameters (OMS 2010) and no female anomalies were included. For each man 2 semen analyses, MSOME (Motile Sperm Organelle Morphology Examination), DNA fragmentation and DNA decondensation were performed. After the oocyte retrieval, the cohort (≥ 8 cumulus-oocyte complex) was shared equally between conventional IVF and IMSI. Embryo transfer was performed at day 2/3 or 5. Variables included embryo quality, implantation, clinical pregnancy and live-birth rates.
Main results and the role of chance: The MSOME performed in all patients was positive (proportion of type 0 and/or type 1 sperm > 8%) in 22 patients (62.9%). By comparing biological parameters, we observed higher embryo cleavage rate and embryo quality rate (top embryo defined with 4 cells at day 2 or 8 cells at day 3, with regular blastomers, < 10% fragments and without multinucleation) after IMSI than after IVF (respectively 36.3% *versus* 16.5%, $p = 0.014$; 12.7% *versus* 4.3%, $p = 0.046$). The number of blastocyst obtained trended to be higher in IMSI (16.7% *versus* 0.0%, $p = 0.081$). No difference was found for fertilization rate and embryo cleavage rate between day 2 and 3. Higher implantation rate was evidenced after IMSI (31.03% *versus* 13.63%, $p = 0.068$). No difference was found for clinical pregnancy rate per transfer. Live-birth rate per transfer was significantly higher after IMSI than after IVF (50.0% *versus* 0.0%, $p = 0.019$).
Limitations, reasons for caution: Small size of study: we need more patients to strengthen our first results. Long time of study-period to include patients.
Wider implications of the findings: Despite the small size of the study, it is suggested that IMSI could improve ART (Assisted Reproductive Technology) parameters in unexplained infertility and failure of IUI.
Trial registration number: Not applied.

P-200 Are smooth endoplasmic reticulum aggregates associated with detrimental outcome?

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Study question: Does smooth endoplasmic reticulum (SER) aggregation negatively impact the overall outcome of ART cycles?
Summary answer: Our findings suggest no evidence that the presence of SER aggregates in the oocyte cytoplasm is associated with a compromised embryological, clinical or neonatal outcome.
What is known already: The presence of SER clusters is considered one of the most severe dysmorphisms, identifiable in metaphase II oocytes, caused by an unknown underlying mechanism. The transfer of embryos originated in these cells has been correlated with an increased risk of abnormal outcome. However, reports of smaller clusters, undetected under light microscopy, found exclusively in presumably SER-negative oocytes but siblings of SER-positive gametes, suggest a compromised cohort of oocytes and possibly an underestimated number of SER-positive transferred embryos. Still, the use of affected gametes and embryos is generally discouraged and should be carried out with caution until its implications are understood.
Study design, size, duration: Retrospective analysis of data regarding 724 ICSI cycles performed between January 2009 and December 2014. Women underwent controlled ovarian hyperstimulation using recombinant FSH or highly purified hMG in either an agonist or antagonist protocol and hCG was administered for the final maturation of the oocytes. Demographic and clinical characteristics of the female population, as well as laboratorial, clinical and neonatal parameters were analyzed.
Participants/materials, setting, methods: Cycles were assorted into two groups, according to the presence or absence of smooth endoplasmic reticulum aggregates in metaphase II oocytes of a population of women 40 years. Group I includes cycles without visible SER clusters and Group II comprises cycles with one or more oocytes with an identifiable SER aggregate. Embryos originated from affected oocytes were selected for transfer exclusively if no other embryos of superior or equivalent quality were available.
Main results and the role of chance: 2815 metaphase II oocytes were checked for the presence of SER aggregates and 128 were affected (4.5%). SER-negative oocytes were found in 642 ICSI cycles and 82 presented at least one cell with SER aggregates (11.3%). Maternal age did not correlate with the presence of SER clusters and the number of retrieved oocytes (8.2 vs. 8.6) and fertilisation rate (65.3% vs. 64.0%) was comparable in both groups. The formation of SER aggregates did not correlate with the stimulation regimen, duration of stimulation, total dose of gonadotrophins, clinical pregnancy rate per embryo transfer (44.9% vs. 39.5%) or miscarriage rate (19.2% vs. 15.6%). The analysis of obstetrical and neonatal data revealed no differences when comparing duration of gestation, birth weight and number of malformations per live birth (5/270 vs. 1/33) in SER-negative and positive cycles. 32 healthy babies were born from the SER-positive group, including 2 pairs of twins originated from a mixed transfer of one SER-positive and one SER-negative embryo.
Limitations, reasons for caution: The frequency, size and location of the cluster in the oocyte was not taken into consideration, and the existence of a threshold for the correlation of these characteristics and a negative outcome cannot be excluded. Also, preferential transfers of embryos originated in SER negative oocytes might be representative of bias.
Wider implications of the findings: Considering that smaller SER clusters may be undetected under light microscopy, the possibility of a higher number of

transfers with affected embryos must be considered. It is fundamental that laboratories record and publish detailed information concerning SER aggregates to better understand its origin and clarify the issues concerning clinical outcome.
Trial registration number: Not applicable.

P-201 Embryological results of couples undergoing ICSI-ET treatments with males currying the single nucleotide polymorphism rs175080 of the MLH3 gene

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Study question: Are there any relationships between the MLH3 gene single nucleotide polymorphism rs175080 of males and the embryological results of couples undergoing ICSI-ET treatments?
Summary answer: The deteriorating effect of the mutant genotype AA on sperm characteristics does not seem to impact on embryo development after fertilization in vitro.
What is known already: The human MLH3 (hMLH3) gene, identified the year 2000, leads to the production of the MutL homolog protein. hMLH3 gene has been suggested to play a role in the DNA mismatch repair mechanism, while it has been also proposed to play a distinct role in the meiotic recombination mechanism. It may be also associated with abnormal spermatogenesis, since a negative impact of the MLH3 rs175080 polymorphism on sperm parameters has been recently found in men. This may affect male fertility, as it has been suggested from the presence of the MLH3 C2531T polymorphism in infertile men.
Study design, size, duration: Our study population comprised of 132 men of couples that were subjected to 132 consecutive ICSI-ET treatment cycles over a 3-year period (2010 to 2012) were included. The 132 men were divided into three groups according to their genotype: the wild type GG ($n = 28$), the heterozygotic type GA ($n = 72$) and the mutant type AA ($n = 32$), while 117 couples finally reached the stage of ET. Fertilization or cleavage failure occurred in 15 of them.
Participants/materials, setting, methods: Participants were men of infertile couples undergoing ICSI-ET treatment. Genomic DNA was extracted from peripheral blood samples from all men followed by conventional quantitative real time PCR for genotyping. Primary endpoint was evaluation of embryological results, while secondary endpoint was pregnancy outcome.
Main results and the role of chance: Significantly lower sperm concentration and progressive motility were observed in the AA group as compared to the other two groups (Concentration: 14.57 + 4.9 mil/ml in AA, 38.3 + 5.4 mil/ml in GA and 41.03 + 6.8 mil/ml in GG, $p < 0.05$, mean + SEM). However, significantly better embryological results (Mean Score of Embryo Quality – MSEQ) were found in the AA (8.12 + 0.5) and the GA group (7.36 + 0.4) as compared to the GG group (5.82 + 0.7), ($p < 0.05$). Clinical pregnancy rate was significantly higher in the AA genotype group (43.8%) and the GA group (30.6%) than in the GG group (14.3%), ($p < 0.05$). Live birth rate was not different.
Limitations, reasons for caution: This polymorphism was studied for the first time. Nevertheless, the number of cases may not allow us to draw solid conclusions. In addition, the possibility that the heterozygotic state of the embryo may be responsible for its competent development cannot be excluded.
Wider implications of the findings: The findings could help in a better understanding of the pathophysiology in human spermatogenesis. In clinical terms, they may provide the means for a better evaluation of the infertile men. Since these are preliminary data, a large prospective randomized trial is needed to study the impact on live birth rate.
Trial registration number: N/A.

P-202 Differences in embryo development after IVM and conventional ICSI assessed by time lapse imaging

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Study question: Is there a difference in the morphokinetics of embryos originating from in vitro matured oocytes as compared to mature oocytes assessed by time lapse Imaging?
Summary answer: Although growth dynamics of embryos after IVM differ from embryos after conventional ICSI, pregnancy rates and live birth rates are similar.
What is known already: In vitro maturation is an established technique in particular in patients with PCOS. However, data on the impact of IVM on embryo development and fetal outcome are rare. Time lapse imaging might be a suitable method to evaluate embryo development after IVM compared to embryos after conventional IVF/ICSI.
Study design, size, duration: Prospective case-control study. Between January 2012 and November 2014 $n = 30$ IVM cycles in $n = 23$ patients with PCOS (G1) were matched to 30 conventional ICSI patients without PCOS (G2) and $n = 19$ patients with PCOS (G3).
Participants/materials, setting, methods: IVM patients were matched according to age and number of oocytes with conventional ICSI patients with and without PCOS. Morphokinetic markers in embryo development like time until pronuclei visibility (tPNa) and fading of pronuclei (tPNf) as well as time to 2- cell (t2) and blastocyst-stage (tB) were analysed in all groups. In addition, cell-cycle lengths, clinical pregnancy and live birth rates were recorded.
Main results and the role of chance: The groups did not differ according to age, BMI and smoking habits. A significant difference was found in AMH levels which were higher in G1 and G3 compared with G2 ($p = 0.000$). In total, $n = 292$ embryos were analysed [$n = 105$ IVM (G1), $n = 115$ conventional ICSI without PCOS (G2) and $n = 72$ with PCOS (G3)]. Embryonal development in G1 was significantly accelerated with regard to tPNa ($p = 0.001$) compared to G2 but slowed down in reaching t6 ($p = 0.032$) and following stages compared to G3. When we focused on the development of only good quality embryos G1-embryos were faster in reaching the first stages compared with G2-embryos (e.g., tPNf, $p = 0.013$). Time till reaching tB was also longer in G1-embryos than in G3-embryos ($p = 0.003$). No significant differences were present with regard to pregnancy and live birth rates between the three groups.
Limitations, reasons for caution: The study population was small, therefore further studies are needed to confirm our results.
Wider implications of the findings: Time lapse imaging may be a useful tool to identify good quality embryos after IVM to enhance pregnancy rates and live birth rates.
Trial registration number: not applicable.

P-203 Euploid rate sensitivity to laboratory culture environment: a blind, prospective, randomised, sibling study

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Study question: To determine whether culturing embryos in two different single step culture media affects the proportion of chromosomally normal embryos.
Summary answer: Following Pre-implantation Genetic Screening (PGS) at the blastocyst stage, change in culture environment was associated with a doubling of the proportion of euploid embryos.
What is known already: Comprehensive chromosomal screening at the blastocyst stage has been proposed as an embryo selection tool and is increasingly widely used for this purpose. Several publications confirm that modern PGS methods accurately distinguish aneuploid embryos from those that are chromosomally abnormal. Female age is the most potent factor influencing embryo aneuploidy rates. Some research has suggested that stimulation protocols might also have the potential to influence embryo ploidy, although this remains controversial. However, the effect of laboratory culture conditions on aneuploidy risk, if any, remains to be established.
Study design, size, duration: A prospective sibling study in a private IVF clinic comparing two single-step culture media with different composition and protein supplementation. Following insemination, 2620 mature oocytes from 363 patients were equally and randomly split between both treatments. Embryologists responsible for grading embryos and deciding on suitability for transfer, cryopreservation or biopsy were blinded to treatment. 333 embryos were

selected for transfer, and genetic information following biopsy was available for 295 blastocysts.

Participants/materials, setting, methods: Medium 1 (KSOM supplemented with complex protein) was compared with Medium 2 (Sage supplemented with Human Serum Albumin, HSA) with regards to traditional Key Performance Indicators (KPIs). Embryos were cultured to the day of transfer/biopsy/cryopreservation in the EmbryoscopeTM. Morphokinetic data were collected similarly to Ciray et al. (2015). Blastocyst biopsies were analysed using Next Generation Sequencing (NGS). Embryos were graded daily (ACE or Gardner grading schemes). Bimodal data were analysed using Chi-square test. Data presented as treatment 1 vs. 2.

Main results and the role of chance: Fertilisation (72% vs. 70%), 3 pronucleate rate (5% vs. 5%), cleavage (99% vs. 99%), blastulation (73% vs. 76%), selection for transfer per MII (12% vs. 13%) and implantation rate (patients under 38 years: 38% vs. 47%; patients under 35 years: 47% vs. 58%) did not significantly differ between treatment. Biochemical loss rate was greater 3.5 fold greater with treatment 1 (23.6% vs. 6.7%, $p < 0.025$), whilst the proportion of chromosomally normal embryos was almost two-fold greater with treatment 2 (16%, $N = 141$ vs. 29%, $N = 154$, $p < 0.025$). The following morphokinetic parameters did not differ between treatments: tPB2, tPNa, tPNf, t2, t3, t4, t5, t6, t7, t8, time of start of compaction (tc), tM, VP, ECC1, ECC2, ECC3, S3, dc, dB, cc2a, cc2b, cc3b, cc3c, tB-tSB, KIDScore median. The following parameters were affected by treatment (tSB: 103 vs. 101 hpi; tB: 110 vs. 108 hpi; teB: 118 vs. 116 hpi; thB: 119 vs. 114 hpi; s2: 1.8 vs. 2.6 h; cc3a: 12 vs. 11 h; tsB-tPNf: 79 vs. 77 h; $p < 0.001$), suggesting that embryos cultured in medium 2 reached the blastocyst stage faster.

Limitations, reasons for caution: The cause of observed differences in blastulation time, biochemical loss and euploidy rate between the two media may not necessarily be directly due to media composition, since, in order to culture both treatments in the same incubator, pH values differed (7.27 vs. 7.14 with incubators set to 7 ± 0.3% carbon dioxide).

Wider implications of the findings: Increased aneuploidy is known to cause reduced implantation, elevated pregnancy loss rate and delayed development, all characteristics of treatment 1. This study suggests that ploidy rates and morphokinetic parameters are sensitive to environmental change, and, therefore, should be monitored as part of the quality control programme in the IVF laboratory.

Trial registration number: Not Applicable.

P-204 Fractal analysis and related forms of complexity of embryo development. Is there a new tool for human embryo selection?

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Study question: Fractal analysis of the microscopic image of embryos, using different forms of complexity, can predict the embryos with highest implantation potential?

Summary answer: We reveal a decrease of fractal dimension from 72 h to 110 h morphokinetics for embryos associated to pregnancy and a constant value for those who not.

What is known already: Fractal analysis has found applications in the detection of coding regions in DNA and measurement of the space-filling properties of tumors, blood vessels and neurons. Fractal concepts have incorporated into models of biological processes, including epithelial cell growth, blood vessel growth and viral infections. There find various uses of fractal geometry in pathology: molecular biology, tumor development, vascular pathology. Three aspects of texture are considered by fractal geometry: fractal dimension, lacunarity and succolarity. The importance of morphokinetics fractal embryo analyse in IVF process reflected in literature is limited. Previous reported data show accuracy prediction of pregnancy between 67.4% and 74%.

Study design, size, duration: The reproductive outcome using fractal dimension analyse provided by EmbryoViewer – Fertilitech, Denmark was analyse by programs ImageJ (IJ) 1.28 and Adobe Photoshop CS4. Fractal dimension was calculated with FracLac for ImageJ using box counting method, and with Fractalyse using grid, mass-radius, correlation and pixel dilation methods. Lacunarity was calculated with FracLac for ImageJ program. Succolarity was calculated using a program we built, on MS VC 2010 Express Edition.

Participants/materials, setting, methods: 168 couples (mean age 36.2 years) was included in the study. Ovarian stimulation was performed with gonadotropins: 87% short antagonist protocol and 13% long agonist protocol, and we did ICSI for 72% and IVF for 28%. We obtained a number of 1092 embryos monitored by time-lapse imaging (EmbryoScope). We include patients where obtain between 3 and 20 oocyte. Transfer was done with single blastocyst to patients who have progesterone level at trigger day below 1.5 ng/dl.

Main results and the role of chance: Fractal dimension was measured by four different methods: mass-radius, box-counting, correlation and pixel dilation. Fractal dimension decrease from 72 to 110 h evolving time (the difference exceeding the standard error) for embryos led to pregnancy and remain constant (linear) for those who did not. For both measured samples, which headed for pregnancy or not, the fractal dimension at 72 h was 1.933 ± 0.002 and 1.932 ± 0.001 respectively, and at 110 h was 1.928 ± 0.002 and 1.930 ± 0.002, respectively. Fractal analysis of the microscopic image of embryo in a culture dish revealed a decrease of lacunarity from 72 to 110 h evolving time, for both embryos which after implant led to pregnancy or not and the lacunarity slope from 72 to 110 h evolving time, decreased only for embryos which led to pregnancy and was constant for those who did not. For both measured samples, which headed for pregnancy or not, the lacunarity at 72 h was 0.197 ± 0.002 and 0.190 ± 0.002 respectively, and at 110 h was 0.142 ± 0.002 and 0.147 ± 0.002, respectively. For both measured samples, which headed for pregnancy or not, the lacunarity slope at 72 h was 0.0725 ± 0.0004 and 0.0675 ± 0.0005 respectively, and at 110 h was 0.0674 ± 0.0003 and 0.0685 ± 0.0003, respectively.

Limitations, reasons for caution: More number of embryo images should use to improve accuracy of prediction model. In this study of embryo morphokinetics we use only images at 72 and 110 h.

Wider implications of the findings: Our results show that fractal model could predict pregnancy in 75%. Using a support vector machine related to supervised learning methods in conjunction with learning based intelligent software could improve pregnancy prediction. In a personalised approach proposed model predicts pregnancy correctly in 83% cases. More study are needed for validation.

Trial registration number: N/A.

P-205 Assessment of the impact of asynchronous syngamy by time-lapse imaging on early human embryo development, implantation and live birth rates

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Study question: Does an asynchronous syngamy (AS) phenotype serve as a primary selection biomarker in predicting embryo development and live birth in human embryos?

Summary answer: AS is associated with higher probability of developing nucleation and cleavage errors. Implantation and live births were not affected in the absence of accompanying errors.

What is known already: With the advent of time-lapse imaging as a non-invasive selection tool for human embryo assessment, the search for additional selection/de-selection parameters, which indicate viability and outcome, has increased. Syngamy as observed by breakdown of pronuclei indicates the union of two sets of chromosomes. This disappearance or pro-nuclei (PN) fading occurs a few hours prior to first cytokinesis. However, an AS phenotype as observed by asynchronicity in the disappearance of pronuclei, indicates a lag in the PN fading pattern and thereby may allow early identification of human embryos with lower development potential.

Study design, size, duration: To assess embryo development and top quality embryos (TQE) based on Istanbul Consensus workshop; 2011, 3141 zygotes cultured in the EmbryoScope ™ between April 2014 and December 2015 were retrospectively analyzed. These were incubated for a minimum of 42 hours to assess their impact on development of nucleation and cleavage errors.

To assess implantation and live birth potential, 2002 transferred embryos cultured between 2011 and 2014 with complete traceability (known implantation data-KID) were included.

Participants/materials, setting, methods: PN fading was annotated for normally fertilized zygotes irrespective of insemination method and user-defined annotation was adopted to denote synchronous and AS phenotypes. The simultaneous disappearance of both the PN was annotated as synchronous syngamy (SS) and was observed by smooth dispersion of nuclear envelopes. However in AS phenotypes, the PNs disappeared one at a time indicating a lag in the fading process. Subsequent annotations of embryo quality were according to the clinic's standard protocol.

Main results and the role of chance: Overall incidence of AS was 15.6% (491/3141) amongst all the embryos analyzed. Of the 3141 zygotes included for embryo development and TQE assessment, AS phenotypes exhibited a higher probability of developing nucleation errors and cleavage anomalies (trichotomous cleavages and rapid cleavages) than those that displayed SS phenotypes (44.8% vs. 38.9%) ($p = 0.02$). Of 3141 zygotes, 28% developed to be scored as TQE. AS zygote phenotypes had a lower probability of developing to TQE, than their synchronous counterparts (22.2% vs. 28.9%) ($p = 0.002$). 2002 transferred embryos cultured under similar culture conditions with regards to media used as well as reduced O2 concentrations, between 2011 and 2014 were included in the outcome study. When AS phenotypes were selected for transfer and their exact traceability known, there was no statistical significance between AS phenotypes and SS phenotypes with respect to KID, (18.4% vs. 20.7%) ($p = 0.45$). Live birth rates were also not statistically significant between AS and SS phenotypes (15.4% vs. 16.5%) ($p = 0.73$). The implantation potential and live birth rates of AS phenotypes with accompanying errors are however underrepresented in the cohort of embryos selected for fresh transfer.

Limitations, reasons for caution: Most of the embryos analyzed were cultured up to day 3 of embryo development and hence any impact on development to blastocyst stage is not known. Confounding factors such as maternal age, etiology of infertility or seminal parameters were not considered. There may be possible selection bias for transferred embryos.

Wider implications of the findings: Observation of AS as early as day 1 of embryo development could serve as novel primary selection biomarker. This along with de-selection biomarkers such as nucleation error and cleavage anomalies observed on subsequent days of development may facilitate identification of viable embryos. Optimizing selection criteria will augment IVF success rates.

Trial registration number: None.

P-206 Time-lapse analysis does not further predict implantation potential of euploid blastocysts in women with advanced maternal age (AMA)

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Study question: Do morphokinetic parameters differ among those euploid blastocysts that will result in clinical pregnancy or not following single embryo transfer in AMA patients?

Summary answer: Euploid blastocysts that result in clinical pregnancy or failed conception have comparable morphokinetic parameters.

What is known already: Several, if not all, studies have reported beneficial effect of various morphokinetic parameters to predict enhanced implantation potential of embryos. However, the ploidy status has not been evaluated in the majority of such studies. In one trial, among patients with ≥2 euploid blastocysts, selection according to the best time-lapse parameters was associated with significantly higher implantation rate when compared to selection with best morphology. However, whether enhanced implantation rate is due to embryo selection or undisturbed culture condition cannot be discriminated. There is no study looking for time-lapse parameters among implanted and not implanted euploid blastocysts cultured in undisturbed condition.

Study design, size, duration: Pre-implantation genetic testing by array-CGH (24-chromosomes) as a routine policy for women with AMA (≥36 yr old), regardless of ovarian reserve, was implemented in April 2015 and included in the current retrospective cohort study. Patients with single gene disorders and translocations were excluded. Of the initial 97 consecutive AMA patients starting controlled ovarian stimulation, 33 had single, euploid blastocyst transfers during April 2015–December 2015.

Participants/materials, setting, methods: All embryos were individually cultured in a time-lapse incubator (Embryoscope, Vitrolife, Denmark) from intracytoplasmic sperm injection up to the blastocyst development. Following trophoectoderm biopsy on Day 5/6, blastocysts were vitrified. A single senior embryologist annotated full morphokinetic information from second polar body appearance up to blastocyst hatching. Single euploid embryo transfer was performed in a frozen replacement cycle in all patients. Clinical pregnancy was defined as visualization of gestational sac with fetal heart beat.

Main results and the role of chance: Of the 33 single, euploid transfers, 23 (69.7%) resulted in clinical pregnancy. Twenty-five time-lapse parameters were analyzed. The mean values of the analyzed 25 parameters were comparable among those blastocysts that implanted or not. Among those 25 parameters, the mean t2 was 26.4 ± 2.5 and 28.4 ± 5.2, in the implanted and non-implanted euploid blastocyst groups, respectively ($p > 0.05$). The respective figures for t3 (38.0 ± 3.2 and 38.2 ± 9.1; $p > 0.05$), t5 (49.1 ± 8.3 and 48.2 ± 12.0; $p > 0.05$), t8 (55.2 ± 8.5 and 57.2 ± 8.2; $p > 0.05$), cc2 (11.1 ± 2.0 and 10.6 ± 3.8; $p > 0.05$), tM (91.9 ± 9.2 and 94.2 ± 14.3; $p > 0.05$), tSB (100.4 ± 7.8 and 105.2 ± 15.2; $p > 0.05$), tB (108.0 ± 7.6 and 114.0 ± 17.3; $p > 0.05$), tEB (112.4 ± 6.6 and 116.5 ± 12.2; $p > 0.05$) and tHB (114.0 ± 7.3 and 118.2 ± 2.8; $p > 0.05$).

Limitations, reasons for caution: Type-II error due to limited sample size cannot be excluded. Lack of automated system for annotation and lack of live birth data are limitations of the current study.

Wider implications of the findings: Time-lapse assessment does not discriminate whether euploid blastocysts will or will not implant following single embryo transfer in a frozen replacement cycle in AMA patients. Further studies are warranted to delineate the euploid blastocyst with the highest implantation potential in such patients.

Trial registration number: None.

P-207 Does morphokinetic assessment predict blastocyst ploidy status?

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Study question: Do time-lapse parameters differ among euploid and aneuploid blastocysts as assessed by trophoectoderm biopsy?

Summary answer: Time to blastulation (tB), time to expended blastocyst (tEB) and time to hatching blastocyst (tHB) are significantly delayed among aneuploid embryos compared to euploid embryos.

What is known already: There are seven studies evaluating the association of time-lapse parameters with ploidy status of embryos with contradicting conclusions. Of these seven studies, cleavage stage-biopsy was performed in three and trophoectoderm biopsy in the remaining four. There is also heterogeneity of the included patient population among the available seven studies. Therefore, there is still no consensus whether time-lapse assessment can be predictive for the ploidy status of embryos as assessed by trophoectoderm biopsy.

Study design, size, duration: In this retrospective cohort study, pre-implantation genetic testing by array-CGH (24-chromosomes) was employed in women with advanced maternal age (≥38 yr old), recurrent implantation failure and recurrent miscarriage. Patients with single gene disorders and translocations were also included. During April 2015 to January 2016, of the consecutive 180 patients enrolled, 107 (59.4%) developed at least 1 blastocyst to be biopsied and 67 (62.7%) had at least one euploid blastocyst. In total, 327 blastocysts were biopsied.

Participants/materials, setting, methods: All embryos were individually cultured in a time-lapse incubator (Embryoscope, Vitrolife, Denmark) from intracytoplasmic sperm injection up to the blastocyst development. Following trophoectoderm biopsy on Day 5/6, blastocysts were vitrified. A single senior embryologist annotated full morphokinetic information from pronuclear formation up to blastocyst hatching. In total, 25 time-lapse parameters were analyzed.

Main results and the role of chance: Of the analyzed 25 time-lapse parameters, 3 were found to be significantly different among euploid and aneuploid blastocysts. Median time to blastulation (tB) [102.58 (range 89.50–125.62)

vs. 108.46 (range 89.06–129.56), $p = 0.01$], time to expanded blastocyst (tEB) [108.31 (range 95.40–129.18) vs. 112.95 (range 102.40–140.06), $p < 0.001$] and time to hatching blastocyst (tHB) [111.65 (range 97.25–139.18) vs. 117.02 (range 103.15–142.32), $p < 0.001$] were significantly delayed among aneuploid embryos compared to euploid ones. In the univariate regression analysis, relative risk for aneuploidy increased with delaying tB (RR = 1.03 95.0% CI 1.00–1.05, $p = 0.027$), tEB (RR = 1.04 95.0% CI 1.01–1.07, $p = 0.003$), tHB (RR = 1.06 95.0% CI 1.02–1.10, $p = 0.002$). However, multivariate logistic regression was not performed due to high level of positive correlation between these 3 time-parameters. When receiver operating curve (ROC) analysis was performed to delineate the optimum cut-off point to predict euploidy, tHB had the best AUC (AUC = 0.77, CI 95%: 0.68–0.87). The optimum cut-off value for tHB was 113.88 (sensitivity 71.7%, specificity 75.7%, Positive predictive value 82.7% and Negative Predictive Value 62.2%).

Limitations, reasons for caution: Embryos from each patient were assumed to be independent observations in the current study, which carries the risk of overestimating potential correlations. However, embryos from one patient may elicit clustering, which means that a large part of the variation observed between embryos can be explained by differences by patients.

Wider implications of the findings: Median time to blastulation, time to expanded blastocyst and time to hatching blastocyst may have limited ability for prediction of aneuploidy.

Trial registration number: None.

P-208 Clinical validation of Eeva test when the embryos are cultured in embryoscope instead of standard incubator

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Study question: To asses how the *Eeva* Test™ algorithm works when embryos are cultured in a benchtop incubator, such EmbryScope™, instead of a standard incubator.

Summary answer: The parameters used by the Eeva™ test to predict embryo viability are a useful tool when implemented as a model in the Embryoscope™.

What is known already: Time-lapse allows for monitoring embryo development, combining morphology with timing of cleavage embryos. In EmbryoScope™, the image acquisition unit is an integral part of the culture chamber, facilitating the monitoring of embryos while they remain inside a controlled, stable culture environment.

Eeva Test™ categorizes embryos on day 3 into "High" or "Low" likelihood of usable blastocyst formation groups using a dark-field, time-lapse imaging and cell-tracking software algorithms. It fits into a standard incubator and although embryos are maintained in a continuous and uninterrupted imaging process from D1 through D3, the conditions are not so stable as in a benchtop incubator.

Study design, size, duration: A retrospective observational cohort study was performed at a University associated private Assisted Reproduction Center between February and November 2015. A total of 103 embryos cultured in Embrysocope™ from 49 patients undergoing an IVF cycle were included in the study. PGD cycles were excluded. Eighteen double embryo transfer and 67 single embryo transfer were performed. Double embryo transfers were included in the study only when transferred embryos had the same Eeva score.

Participants/materials, setting, methods: All the embryos were cultured in the Embryoscope™ and scored as "High" or "Low" with the option "Compare and select" of the EmbryoViewer software using a model based on the Eeva™ Test. The model took into account the timings for P2 (the time between the first and second cytokinesis) and P3 (the time between the second and the third cytokinesis) that are the parameters used for developing Eeva prediction and cell-tracking software.

Main results and the role of chance: The mean number of transferred embryos was 1.21. Eighty out of the 103 embryos transferred were scored as high ($9.33 \geq P2 \geq 11.45$ h and $0 \geq P3 \geq 1.73$ h) whereas 23 were scored as low (P2, P3 or both, out of the Eeva High window). Overall implantation rate was 37.86%: 42.42% and 35.09% for those scored as high and low respectively ($t = 1.044$; $p = 0.298$). The clinical pregnancy rate in those high scored embryo transfers was 48.49% whilst a percentage of 35.09% was the achieved clinical pregnancy rate among the low scored embryo transfers (Pearson`s Chi square = 3.204; $p = 0.073$). Finally we found both clinically and statistically significant differences in ongoing pregnancy rates between high and low scored embryo transfers (42.42% and 21.05% respectively; Pearson`s Chi square = 8.612; $p = 0.003$). There were no significant differences in age between the patients that got pregnant and those who did not. Our embryo implantation and pregnancy rate data correlate to those published in the literature when the Eeva™ Test is used to score embryos in a standard incubator. Moreover, our data confirm a positive and significant association between the high scored embryo transfers and a higher ongoing pregnancy rates.

Limitations, reasons for caution: Limitation is due to the subjectivity when choosing the exact time of cell division in the Embryoscope. P2 and P3 are narrow windows that the Eeva software detects automatically and objectively, because of that, the embryologist should be consistent with himself and we the rest of embryologist in the lab.

Wider implications of the findings: The advantages of undisturbed embryo culture in a stable device such as the EmbryoScope™ time-lapse incubator in association with the use of the model of *Eeva* Test™ could represent an important improvement in IVF, reducing early pregnancy loss and increasing ongoing pregnancy and implantation rate.

Trial registration number: No clinical trial.

P-209 Effect of antioxidants addition on the redox sate in vitrified/warmed human oocytes: preliminary results

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Study question: Does the Crocin, a natural carotenoid antioxidant from saffron, decreases the oxidative stress in vitrified human oocytes?

Summary answer: Our data show that the intracellular oxidation state can be decreased by adding the antioxidant Crocin in the vitrification/warming media.

What is known already: It is well known that vitrification can cause subtle damage in oocytes. One of the most important aspects to consider is the alteration of the normal mitochondrial function that could be responsible of the increase of oxidative stress in the cryopreserved female gametes. Previous studies from our group demonstrated that oocyte vitrification produces an increase of the oxidative stress in young women (<27 years old) when compared with fresh oocytes. Ooplasmic redox state was evaluated in vivo by measuring FAD++/NAD(P)H autofluorescence ratio, which is a marker of the intracellular redox condition.

Study design, size, duration: Case control study including a total of 78 oocytes (control group = 34, treatment group = 44,) from young oocyte donors (average age 25.57 ± 4.45) recruited from December 2014 to October 2015. Oocytes were vitrified and warmed with the antioxidant Crocin and without antioxidant (control group).

Participants/materials, setting, methods: After signed informed consent, 25 patients undergoing controlled ovarian stimulation donated their discarded oocytes to research. Thirty four oocytes were vitrified and warmed without antioxidant (control group) and forty four oocytes were vitrified and warmed with 400 µg/mL of the Crocin (treatment group). Intracellular redox state was assessed by measuring NAD(P)H and FAD++ autofluorescence ratio by confocal microscopy. Statistical analysis was performed using Student's *t*-test for means comparison. *p*-values 0.05 were considered statistically significant.

Main results and the role of chance: We found that both oocyte groups, control and treatment, showed similar vitrification survival rates (88.09% vs. 85.45% respectively, *p*-value >0.05). Confocal microscopy revealed that the FAD++/NAD(P)H ratio was higher in conventional vitrified oocytes than in oocytes vitrified/warmed with the antioxidant (0.76 ± 0.23 vs. 0.60 ± 0.16; *p*-value < 0.05). This result shows a decrease in the oxidation state in donor oocytes when adding 400 µg/mL of the antioxidant Crocin in the vitrification/warming media.

Limitations, reasons for caution: Additional studies with a larger number of oocytes are required to confirm these preliminary results.

Wider implications of the findings: Our results suggest the oxidative stress of human oocytes is decreased following the addition of the antioxidant Crocin in the vitrification/warming media, which eventually may improve the embryo

development. Our findings emphasize the relevance of continuing research with antioxidant molecules, in order to obtain better outcomes after oocyte vitrification procedures.
Trial registration number: NA.

P-210 High gonadotropin dosage does not affect euploidy and pregnancy rates in PGT cycles

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Study question: Does high gonadotropin dosage affect euploidy and pregnancy rates in PGT cycles? The purpose of this study was to compare euploidy and pregnancy rates after administration of different dosages of gonadotropins in PGT cycles.
Summary answer: High gonadotropin dosage does NOT affect euploidy and pregnancy rates in PGT cycles.
What is known already: The proportion of euploid embryos in PGT (Preimplantation genetic testing) cycles decreases significantly with advancing maternal age. Recruitment of an adequate number of follicles is crucial in PGT cycles and it can be regulated by administration of proper gonadotropin doses.
Study design, size, duration: 406 cycles of IVF treatment with PGT between January 2013 and November 2015 were included in the study (average number of embryos for ET – 1.17). In 132 IVF cycles (109 patients, average age – 35.37 ± 2.21) less than 3000 IU were administered, in 196 IVF cycles (153 patients, average age – 37.79 ± 3.01) from 3000 to 5000 IU were administered and in 78 IVF cycles (59 patients, average age – 39.67 ± 4.19) over 5000 IU were administered.
Participants/materials, setting, methods: A retrospective study of SNP PGT outcome data was conducted to identify differences in euploidy and clinical pregnancy rates. Patients were divided into three gonadotropin dosage groups (<3000 IU, 3000–5000 IU, >5000 IU per IVF cycle) and in four age groups (<35, 35–37, 38–40, ≥41 year old).
Main results and the role of chance: Euploidy rates in the young patient group (<35 year old) were similar regardless of gonadotropin dosage used during controlled ovarian stimulation: 59.66% in the group of patients with low gonadotropin dose (<3000 IU), 62.79% in the group of patients with medium dose (3000–5000 IU), and 66.67% with high gonadotropin dose (>5000 IU). The difference in euploidy rates between the three gonadotropin dosage groups was not statistically significant: $p = 0.5$, $\chi^2 = 0.454$. In the group of patients 35–37 years old euploidy rates ranged from 53.1% to 60.01% ($p = 0.253$, $\chi^2 = 1.31$), in the group of patients 38–40 year old euploidy rates ranged from 43.28% to 51.11% ($p = 0.283$, $\chi^2 = 1.153$) and in the group of patients over 40 year old euploidy rates ranged from 28.57% to 32.26% ($p = 0.623$, $\chi^2 = 0.238$). Ongoing pregnancy rates were statistically not different within all age groups (ranging from 55.22% to 60.79%) ($p = 0.422$, $\chi^2 = 0.645$).
Limitations, reasons for caution: Retrospective study and heterogeneity of patients included.
Wider implications of the findings: Analysis of the data proved that euploidy and ongoing pregnancy rates are not affected by high gonadotropin dosage in PGT cycles. To maximize efficiency and cost-effectiveness in PGT cycles the number of follicles recruited in each cycle should be limited only by medical safety concerns and patient well-being.
Trial registration number: None.

P-211 Inter-observer agreement between embryologists during selection of a single blastocyst for transfer: a multicenter study

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Study question: What is the inter-observer variability between embryologists when selecting a blastocyst for transfer on day 5?
Summary answer: A good level of agreement was found between embryologists in all assessments evaluated.
What is known already: One of the major factors for pregnancy achievement after IVF is embryo quality. The process of determining embryo quality, though, is highly subjective because it involves the interpretation and application of certain morphological criteria by individual embryologists. Some variation in embryo scoring is therefore expected among different assessors and may compromise the accuracy of morphology-based embryo selection. Previous research on the morphological assessment of early-stage embryos has shown varying levels of inter-observer agreement. However, single blastocyst transfer is now becoming increasingly popular and there is no published data that assesses inter-observer agreement when selecting an embryo for day 5 transfer.
Study design, size, duration: This was a prospective study performed using ten embryologists working at five different clinics within IVFAustralia in New South Wales between July 2013 and November 2015. These embryologists were selected based on their yearly QAP (Quality Assurance Program) results for blastocyst grading and were asked to choose an embryo for transfer in 100 cases using 2D images obtained from an EmbryoViewer.
Participants/materials, setting, methods: Cases with ≥2 Day 5 embryos were included in this study. A questionnaire was developed with a web survey designer. For each case, day 5 images (one for every embryo) were shown and embryologists were asked to make a decision for transfer for each case. Subsequently, day 3 and day 5 images of the same embryos were shown and embryologists' were asked to decide again. Inter-observer agreement was assessed using the kappa coefficient.
Main results and the role of chance: Inter-observer agreement among embryologists when selecting an embryo for transfer on day 5 was good (kappa = 0.734, 95% CI 0.665–0.791). This agreement was similar in the subgroup analyses performed according to professional experience (≤ 10 years vs. >10 years: kappa = 0.745, 95% CI 0.660–0.811 vs. 0.735, 95% CI 0.677–0.806), research experience (no vs. any research experience: kappa = 0.716, 95% CI 0.629–0.776 vs. 0.748, 95% CI 0.696–0.814) and exposure to embryo grading (≤ 2 days vs. ≥3 days per week: kappa = 0.743, 95% CI 0.677–0.819 vs. 0.736, 95% CI 0.676–0.794). Similarly, the agreement between embryologists was good when there was more than one top quality embryo (kappa = 0.745, 95% CI 0.667–0.871) or when there were only poor quality embryos available for transfer (kappa 0.718, 95% CI 0.648–0.775).

The agreement between embryologists, when day 3 images were added, was still good, but slightly decreased (kappa = 0.676, 95% CI 0.617–0.724), which was also seen throughout in every subgroup analyses. This was also observed when there was more than one top quality embryo (kappa = 0.674, 95% CI 0.574–0.802) or when there were only poor quality embryos available for transfer (kappa = 0.637, 95% CI 0.420–0.841).
Limitations, reasons for caution: All embryologists had already fulfilled their training and were working under one organisation with similar policies. The inter-observer agreement might not be as high between embryologists with different levels of training or between embryologists working in clinics with different policies.
Wider implications of the findings: Although the agreement between embryologists was deemed good for most of the outcomes of this study, it could certainly be further improved leading potentially to more consistent pregnancy rates. Future studies need to be directed towards methods or technologies that can help achieve this.
Trial registration number: N/A.

P-212 Elective vs. non-elective fresh Single Blastocyst Transfer (SBT) in women aged 35–40 years

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Study question: Does the availability of blastocysts for vitrification correlate with higher pregnancy rate compared to cycles without vitrified blastocysts in fresh single blastocyst transfer (SBT) in women aged 35–40.
Summary answer: In women aged 35–40 years, fresh elective SBT (eSBT) achieves higher pregnancy rate compared to fresh SBT cycles without supernumerary vitrified blastocysts (obligatory SBT)
What is known already: Availability of supernumerary embryos for cryopreservation is known to be associated with higher implantation rates in cleavage-stage embryo transfers. It has also been shown that in young, good

prognosis patients, eSBT correlates with better implantation rate compared to obligatory SBT. Data on eSBT in women aged 35 years and over are scarce. As this group of patients is more prone to adverse perinatal outcome in case of multiple pregnancy, finding prognostic markers for clinical pregnancy which can promote the practice of single embryo transfer are of paramount importance.

Study design, size, duration: A retrospective cohort study performed at a single academic reproductive center between January 2012 and June 2015. All cycles with a fresh autologous SBT of good quality embryos (defined as Gardner's grade ≥3bb) were included in the analysis. Exclusion criteria were cycles with SBT of fair and poor quality blastocysts, multiple embryos transferred, cycles using donor oocytes, frozen-thaw embryo transfers and cleavage-stage embryo transfers.

Participants/materials, setting, methods: There were 692 fresh, autologous, SBT cycles of good quality blastocysts in women aged 35–40 years available for analysis. All embryos were cultured to the blastocyst stage and transferred on day 5. According to provincial guidelines single embryo transfer is mandatory in this age group, so excess blastocysts of adequate quality were vitrified on day 5 or 6. Comparison between study groups was performed by *t*-test and Chi-square tests where applicable.

Main results and the role of chance: In 555 fresh SBT cycles, supernumerary blastocysts were available for vitrification (80.2%). In these cycles elective SBT was performed and the rest of the blastocysts were vitrified. An average of 2.8 ± 1.8 supernumerary blastocysts were vitrified. In 137 fresh SBT cycles no blastocysts were available for vitrification (obligatory SBT). Mean age of patients was similar in both groups (36.7 ± 1.3 vs. 36.8 ± 1.3 years, $p = 0.51$). Number of MII oocytes was significantly higher in the elective SBT group compared to the obligatory SBT group (9.6 ± 4.6 vs. 6.97 ± 4.4, $p < 0.01$) although fertilization rates were similar (81.3% in the eSBT vs. 78% in the obligatory SBT, $p = 0.38$). Clinical pregnancy rate per transfer (defined as intra-uterine fetal heart rate by vaginal ultrasound at 6 weeks) was significantly higher in the eSBT group compared to the obligatory SBT group (46.1% vs. 35%, $p = 0.01$).

Limitations, reasons for caution: Fair to poor quality blastocysts were excluded from the study in order to control for transferred embryo quality and avoiding a bias against the obligatory SBT group. This may suggest the results may not apply to poor prognosis patients. Also, the retrospective nature of the study limits conclusions.

Wider implications of the findings: Similar to younger patients, eSBT achieves higher pregnancy rates in women aged 35–40 years. This may imply that supernumerary blastocysts may serve as positive prognostic markers and promote SBT in this age group. This practice may reduce multiple pregnancy risk in these patients who are candidates for multiple embryo transfer.

Trial registration number: none.

P-213 The availability of time-lapse (TL) analysis proves the relationship between morphokinetics and outcomes in IVM with PCOS

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Study question: Is it effective using kinetic information based morphokinetic evaluation for embryo selection in IVM with polycystic ovaries syndrome (PCOS)?

Summary answer: With the exclusion of abnormal events in development simultaneously, kinetic information could offer new additional technique in embryo selection for transfer in IVM with PCOS.

What is known already: TL analysis by morphokinetic monitoring of embryonic development is safe and reliable embryo selection method. Lots of studies suggest that selection of embryos with detailed observation using TL system could improve pregnancy rates in IVF. But, there are few studies examining whether the possibility of benefit from TL in IVM and the effect on clinical outcomes of IVM is still under debate.

Study design, size, duration: This retrospective study was conducted from August 2013 to August 2015. It included 148 infertile patients. Embryos were incubated in TL incubator (EmbryoScope™) and morphokinetics of 820 embryos from oocytes matured in vivo or in vitro was analyzed.

Participants/materials, setting, methods: The average age was 32.0 ± 1.6 and 32.7 ± 3.0 and the total number of embryos after ICSI was 412 and 408 for in IVF and IVM, respectively. We compared clinical outcomes and timepoints of each morphokinetic events and abnormal events between IVF and IVM.

Main results and the role of chance: There were no significant differences in the age and the number of retrieved oocytes between two groups. Although fertilization and good quality embryo rates were lower in IVM (54.0% vs. 67.5% and 18.4% vs. 52.2%, $p < 0.0001$, respectively), implantation rate (22.5% vs. 28.9%) was not differ.

All the morphokinetic parameters were analyzed. Compared to IVF embryos, extrusion of second polar body was slower (3.7 h vs. 3.1 h, $p = 0.024$) and existence of pronuclear was shorter (14.0 h vs. 14.3 h, $p = 0.025$) in IVM. No significant differences were observed between the time-point of pronuclear fading and t5.

In IVM embryos, the rates of direct cleavage (<5 h) and the incidence of multi nucleated blastomere and embryo arrest were higher significantly (32.6% vs. 22.8%, $p = 0.002$, 46.3% vs. 34.9%, $p < 0.001$, 8.8% vs. 4.4%, $p = 0.01$, respectively).

Limitations, reasons for caution: This retrospective study may not represent all embryos because this includes small number of oocytes. Small data size is a limiting factor to make reliable statistical analysis and sample size needs to be enlarged.

Wider implications of the findings: Embryo selection for transfer by morphokinetic analysis and detection of abnormal development may provide an opportunity for improving the success rate in IVM cycle. In addition our study could confirm previously studies about embryos generated from IVM have more increased rate of abnormal development by TL system.

Trial registration number: NA.

P-214 Predictive model to determine the optimal number of fresh versus vitrified oocytes to obtain similar implantation and live birth rates in a donor oocyte program

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Study question: Which donor and recipient factors are relevant to predict implantation and live birth rate in an egg donor program with fresh versus vitrified oocytes?

Summary answer: The predictive model included seven factors: gamete source (fresh vs. frozen/vitrified), sperm concentration, donor´s fertility, donor's estradiol (E2) at trigger, donor and recipient body mass index (BMI).

What is known already: Currently there are predictive models that analyze clinical and embryo parameters to predict clinical outcomes after IVF, e.g., risk of multiple pregnancies. However, there are no reports of models to determine the optimal number of oocytes needed for egg donation with fresh and vitrified oocytes. Survival rates in oocytes vitrified are not 100%. Addition, vitrified oocytes yield blastocyst formation that are less than cycles using fresh donor oocytes. Thus, the number of vitrified oocytes needed in an oocyte donation cycle is likely higher than a fresh donor cycle.

Study design, size, duration: We performed a retrospective observational study with oocyte recipients presenting for treatment at IVI Santiago de Chile during the years 2012–2014. The study includes 645 cycles of oocyte donation, 462 fresh and 183 vitrified oocyte cycles from which a total number of 9583 oocytes were included.

Participants/materials, setting, methods: Econometric modeling was applied to build a model capable of providing an estimate for the appropriate number of fresh or vitrified oocytes needed in a recipient cycle. The dependent variables studied were all discrete. When possible, we used multinomial logit models and otherwise we used generalized lineal models representing a binomial process with a logit link function. Variable significance was analyzed through a *t*-test.

Main results and the role of chance: With the data analysis, seven parameters were determined important for development of the algorithm: donor and recipient BMI, gamete origin (fresh or frozen/vitrified), sperm concentration, E2 at trigger and fertility of donor (previous pregnancy from donation). According to our data, more vitrified oocytes are needed to achieve similar rates of implantation and live birth compared to fresh oocytes. Using the algorithm we may calculate the additional number needed on vitrified oocytes cycles. That number

it depends on the clinical characteristics and parameters of the cycle of donors and patients considered in the model.

The impact of oocyte vitrification is illustrated by the following example: while keeping other parameters constant, four fresh versus five vitrified oocytes are required to obtain an implantation rate of 61.45% with a confidence of 70%. This algorithm allows a user to enter data for the likelihood of implantation and live birth based on number of oocytes.

Limitations, reasons for caution: Not all factors that impact success of IVF were included. In addition, the correlations identified may vary in different populations or countries.

Wider implications of the findings: A predictive model capable of estimating the probability of implantation and live birth would be extremely helpful to improve outcomes and assist clinicians deciding how many oocytes are needed in a oocyte donation programs, according to the odds to get an blastocyst and live birth for each mature oocyte.

Trial registration number: not trial study.

P-215 Antinuclear antibody of discrete speckled immunofluorescence staining pattern is associated with polypronuclear fertilization

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Study question: The present study was conducted to investigate the influence of immunofluorescence staining pattern and titer of antinuclear antibody (ANA) on maturation and fertilization of human oocyte.

Summary answer: Maturation rates were similar between ANA-negative and positive irrespective of immunofluorescence staining pattern. Polypronuclear fertilization increased significantly in ANA of discrete speckled pattern.

What is known already: ANA is an autoantibody against nuclear components of eukaryotic cells such as DNA, centromere and histone. Immunofluorescence staining pattern of ANA is commonly applied to identify corresponding antigens. Discrete speckled staining pattern indicates that its corresponding antigen is centromere. The relationship of anti-centromere antibody (ACA) with abnormal oocytes maturation, compromised embryo developmental competence and poor prognosis post pregnancy in human IVF has been reported.

Study design, size, duration: Total of 1646 cycles of IVF (355 cases with ANA determined) from January 2012 to December 2013 were retrospectively investigated.

Participants/materials, setting, methods: Patients with positive ANA were divided into 4 groups according to their patterns (A: homogeneous, B: speckled, C: nucleolar, D: discrete speckled) and ANA negative patients were used as control. The rates of oocytes maturation and polypronuclear fertilization either by IVF or ICSI were compared among its patterns and control. Patients with discrete speckled pattern were further divided into 5 groups based on their titer. Correlation between high polypronuclear fertilization rate (>25%) and antibody titer was also analyzed.

Main results and the role of chance: The rates of maturation, polypronuclear fertilization in IVF and polypronuclear fertilization in ICSI of control were 75.9% (3772/4870), 9.0% (72/798) and 4.6% (137/2974), respectively. Rates of group A were 80.3% (965/1201), 11.7% (33/282) and 4.0% (27/683), respectively. Rates of group B were 82.6% (2037/2465), 11.7% (58/495) and 4.3% (66/1542), respectively. Rates of group C were 94.6% (210/232), 7.4% (8/108) and 4.9% (5/102), respectively. Rates of group D were 84.2% (85/101), 50.0% (27/54) and 51.6% (16/31), respectively. Maturation rates were similar between ANA-negative and positive regardless of staining pattern. The ANA-positive patients with discrete speckled pattern showed significantly higher incidence of polypronuclear fertilization both in IVF and ICSI than control ($p < 0.01$; x2-test). Percentages of the patients with high polypronuclear fertilization rates in the group of antibody titer 1:80, 1:160, 1:320, 1:640 and 1:1280 were 100.0% (2/2), 100.0% (3/3), 0.0% (0/1), 0.0% (0/1) and 90.9% (10/11), respectively. No correlation was confirmed between high polypronuclear fertilization rate and intensity of antibody titer.

Limitations, reasons for caution: Discrete speckled staining pattern indicates its corresponding antigen is centromere. However, evaluation of staining pattern is not well established.

Wider implications of the findings: Polypronuclear fertilization increased in the patients with discrete speckled pattern of ANA. This result suggests that ACA might cause polypronuclear fertilization and further research is required to clarify the mechanism. Embryos for transfer should be carefully selected for the patients with positive ANA, especially with discrete speckled staining pattern.

Trial registration number: None.

P-216 Fatty Acid-Binding Protein 4 in endometrial epithelium is involved in embryonic implantation

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Study question: What are the roles of fatty acid-binding protein 4 of endometrial epithelial cell in the establishment and maintenance of pregnancy and early pregnancy loss?

Summary answer: FABP4 regulates embryo implantation via altering uterine receptivity, and decreased expression of FABP4 in endometrium may be linked with pregnancy loss.

What is known already: The establishment and maintenance of pregnancy occurs through the interaction between maternal uterine endometrium and embryo (mainly the trophoblasts). In the research before, we found FABP4 is an important regulator for the proliferation, migration and invasion of endometrial epithelial cells.

Study design, size, duration: Cell experiment, animal experiment and molecular biology experiment.

Participants/materials, setting, methods: The expression of FABP4 and uterine receptive factor was determined by western blotting. FABP4 siRNA and FABP4 inhibitor were used to silence FABP4 and the function of FABP4 respectively. ICR mice and trophoblast spheroids mimicking embryos were set up to evaluate the effect of FABP4 silence or inhibition on embryo implantation in vivo.

Main results and the role of chance: The expression of FABP4 mRNA was significantly decreased in the deciduas of women with early pregnancy loss compared with that of women with normal pregnancy ($p = 0.04$). FABP4 siRNA significantly reduced the number of embryos implanted ($p < 0.001$) and FABP4 expression in ICR mice. FABP4 inhibition also significantly decreased the number of embryos implanted ($p < 0.001$). Either silence or inhibition of FABP4 in endometrial epithelial cell abolished the expression of uterine receptive factors induced by the combination of estrogen and progesterone, and reduced the number of trophoblast spheroids adhered onto endometrial cell.

Limitations, reasons for caution: The limitations of this study include relative small size of women with pregnancy loss was recruited and embryonic aneuploidy was not excluded in women with EPL.

Wider implications of the findings: FABP4 regulates embryo implantation via altering uterine receptivity, and decreased expression of FABP4 in endometrium may be linked with pregnancy loss, indicating FABP4 has biological role in the establishment and maintenance of pregnancy and subsequently is involved in pathogenesis of pregnancy loss.

Trial registration number: Natural Scientific Foundation of China (81170572).

P-217 Asymmetric division in the first cleavage of human fertilized oocytes observed by high-resolution time-lapse cinematography and its negative impacts on further developmental potential

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Study question: Does asymmetric division occurring in the first cleavage of human fertilized oocytes adversely affect their further developmental potential?

Summary answer: Asymmetric division in the first cleavage leads to poor developmental potential, suggesting the first cleavage pattern might be a useful parameter predicting further embryonic development.

What is known already: Predicting embryo viability reliably and safely is crucial to the successful application of assisted reproductive technology (ART). To this end, we have found various novel phenomena correlated with embryo quality and have clarified the velocity of human embryonic development by high-resolution time-lapse cinematography (hR-TLC). However, there is still

no reliable benchmark to assess future embryo quality. According to the Istanbul consensus (2011), both the time course and morphology of human embryonic development are important for assessing subsequent embryo quality; however, this study did not consider the impact of morphokinesis events such as cell-volume asymmetry between blastomeres.
Study design, size, duration: Since 2003, we have used donated oocytes ($n = 230$) for hR-TLC observation. Of those, 157 were fertilized normally and 86 developed to the 2-cell stage. We calculated the maximal cross-sectional area at the 2-cell stage just after the first division, and thus categorized the 2-cell embryos into two groups (symmetric; area difference ≤ 800 μm^2, asymmetric; area difference >800 μm^2). We then compared further embryonic development using hR-TLC between symmetric and asymmetric embryos.
Participants/materials, setting, methods: Of 157 fertilized oocytes, 63 were fertilized by conventional in vitro fertilization (c-IVF) and 94 by intracytoplasmic sperm injection (ICSI). The hR-TLC observation was commenced 1 hour post-insemination by c-IVF, and immediately after ICSI procedures. Images were acquired for 50 ms (exposure time) at 2-minute intervals for approximately 40 h. Once the hR-TLC imaging was complete, good-quality embryos developed to the 4-cell stage were subsequently cryopreserved for future clinical use.
Main results and the role of chance: Of the 157 fertilized oocytes, 86 (IVF; $n = 34$, ICSI; $n = 52$) cleaved into two cells at the first cleavage, and of those 2-cell embryos, 69.8% (IVF; $n = 25$, ICSI; $n = 35$) cleaved symmetrically, and the remaining (IVF; $n = 9$, ICSI; $n = 17$) cleaved asymmetrically. The rate of multinucleated blastomeres in the asymmetric group (50.0%; 13/26) was significantly higher than that in the symmetric group (23.3%; 14/60; $p < 0.05$). The rate of good quality embryos after the second cleavage was also significantly higher in the symmetric group at 63.3% (38/60) than in the asymmetric group at 15.4% (4/26); $p < 0.01$). Embryos cleaved in an asymmetric manner at the first cleavage thus showed a higher rate of multi-nucleation and poor development, while there was no significant difference in the patient profiles or time course of each embryonic morphological event between the symmetric and asymmetric groups.
Limitations, reasons for caution: Our findings suggested that asymmetric division might be involved in aberrant spindle position or cytoskeletal abnormalities during human embryonic development. However, further studies including molecular biological analyses are needed to clarify the mechanisms leading to this phenomenon.
Wider implications of the findings: We showed that embryonic development was significantly better in 2-cell embryos with a smaller cross-sectional area difference between cells (≤ 800 μm^2) than in those with >800 μm^2 difference between cells, suggesting cytoskeletal dysfunction. This parameter would be useful for detailed assessments of human embryos by the Istanbul Consensus.
Trial registration number: None.

P-218 Cytokine GM-CSF in day 3 spent culture drops decides the fate of embryonic development to blastocyst stage & quality of blastocyst

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Study question: Quantitative estimation of GM-CSF in day 3 spent culture media drops to investigate whether early human embryos cultured in-vitro synthesize their own GM-CSF and utilize it for their development into blastocyst.
Summary answer: Utilization of GM-CSF produced endogenously by early human embryo influences blastocyst formation rate and quality in terms of ICM development and number of TE cells.
What is known already: GM–CSF expressed in the female reproductive tract, is produced by epithelial cells of structures such as the ovaries, fallopian tubes, endometrium and pre-implantation embryos. In humans and mice, GM-CSF receptor has been detected in embryos from the fertilized oocyte until the blastocyst stage. GM-CSF, due to its anti-apoptotic and mitogenic effects, is known for its importance in the development of blastocysts and in normal fetal and placental development. Several studies have concentrated on embryonic development in culture media supplemented with GM-CSF. But none has focused on influence of endogenously synthesized GM-CSF by embryo on embryo development.
Study design, size, duration: Prospective study without randomization done at our IVF centre from May 2015 to October 2015. In every IVF cycle, each fertilized zygote was cultured individually in cleavage media drop till day 3. Neither the media contained GM-CSF nor was the culture drop externally supplemented with GM-CSF. After shifting all embryos ($n = 203$) into blastocyst medium, GM-CSF in each of the day 3 spent drops was measured by ELISA. Quality of Day 3 embryos and day 5 blastocysts was morphometrically evaluated.
Participants/materials, setting, methods: According to the 25 and 75 percentile values of GM-CSF in spent culture drops, three groups Low (<3.75 pg/ml; $n = 50$), Medium (3.75–9.78 pg/ml; $n = 97$) and High (>9.78 pg/ml; $n = 56$) were formed. All day 3 embryos and day 5 blastocysts were evaluated morphometrically and graded as Top/Good/Poor quality as per Gardner's criteria. Blastocyst formation rate and quality of blastocysts formed were the main outcome measures. Statistical analysis was done using Graph-pad Prism V software.
Main results and the role of chance: Age of patient remained comparable in all three groups with no statistically significant difference (One way ANOVA $p = 0.3128$). However, D3 embryo quality was significantly better in low compared to medium & high GM-CSF content groups ($p = 0.0398$). Blastocyst formation rate was extremely significantly high in low GM-CSF as compared to medium/high GM-CSF groups ($p = 0.0008$). Blastocyst quality also showed significant differences between the three groups. Blastocoelic cavity was much superior and larger ($p = < 0.0001$); organization and compactness of inner cell mass (ICM) was greater ($p = 0.0015$); and the number of trophectodermal (TE) cells was significantly higher ($p = 0.0004$) in the low GM-CSF group than in the medium/high GM-CSF groups. Interestingly, no significant difference in these parameters was seen when intercompared between just the medium and high GM-CSF groups. The correlation of content of GM-CSF in all spent drops with blastocyst formation and its quality was inversely proportional with a robust correlation coefficient (Spearman $r = – 0.2188$; $p = 0.0017$). Hence, this unique study successfully implicates GM-CSF level in day 3 leftover culture medium drop (after transferring embryos to blastocyst culture medium) in formation of higher number and better quality of blastocysts.
Limitations, reasons for caution: To our knowledge this is the only study that explores the possibility of endogenous GM-CSF production by embryo for its development. Hence larger multicentre studies are needed to endorse these findings. It is also imperative to establish why and how poor quality embryos extrude un-utilized GM-CSF in the culture media.
Wider implications of the findings: Concept of GM-CSF supplementation to culture media for better embryonic development is gaining ground in recent times. We believe, it would be appropriate to follow natural pathway and add GM-CSF only to select embryos that have high GM-CSF content in day 3 culture drops rather than empirically adding to all embryos.
Trial registration number: Not Applicable.

P-219 Determination of morphological variables during embryo culture that explain the human blastocyst development through model selection algorithms

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Study question: Which human morphological variables during embryo culture explain the successful development of a day 5 embryo based on model selection algorithms?
Summary answer: Model analysis showed that Fertilization method, third day symmetry, number of cells and fragments at fourth day, are variables that can explain the successful development.
What is known already: After fertilization, morphological evaluation criteria are used daily during embryonic development, mainly based on Veeck and recently, morphokinetics apparently improves this evaluation. Embryo selection parameters such as the fertilization method, pro-nucleous morphology, polar

bodies morphology, cleavage time, blastomere organization, presence or absence of citoplasmatic fragments, are among the most important criteria. These embryo features related to morphology can indirectly explain the appropriate function of the intracellular machinery responsible of implantation process. This is relevant because it has been suggested that aberrant development in the early stages can impact negatively in later stages of embryo development.
Study design, size, duration: A retrospective analytical study, assessed with computational statistical analysis using R programming software. Database included observations from 2013–2014. All records were performed by the same embryologist and per duplicate. Statistical analysis was performed to obtain multiple interactions between 18 embryo morphological variables in 679 entries.
Participants/materials, setting, methods: Collected database of embryo morphological assessment generated in ART using standardized protocol based on Veeck criteria. Sample consists of embryos transferred on day 3 and 5, frozen in day 3 and 5 or blocked in days 3, 4, 5. Observations were recorded during five days. Patients ranged from 26–45 years. Variables for this study were fertilization, Z score, cells from Days 2–5; fragments amount and disposition through 2–5 days. Symmetry and organization at days 2–5.
Main results and the role of chance: A series of mathematical models were proposed, whose structure was determined using a formula generator which considered all possible combinations of the predictor variables. Logistic regression models were adjusted and parameter values were estimated. A hypothesis test of all parameters for each logistic regression was performed and the models with significant adjustment were chosen. Finally, a comparison of the models performance using the AIC (Akaike Information Criterion, which is a maximum likelihood criterion used in the selection of statistical models, which penalizes models with more parameters). Therefore the model with the lowest AIC was chosen. It was generated formulas for each of the variables and their possible combinations using the model selection algorithm and we found that the best combination of variables were (Fertilization method, third day symmetry, fourth day developmental stage, and fourth day fragmentation with a value of AIC = 362.49.
Limitations, reasons for caution: Morphological criteria has limitations to predict embryo clinical outcomes, because depend on the embryologist criteria. The variables included in this study were numerical and categorical; however, other variables could be included to improve the power of the analysis and the predictive value such as metabolic or genetic markers information.
Wider implications of the findings: The results of this advanced data analysis could help to determine which of the evaluated variables and combinations have the potential to explain the success based on embryo morphological observations. This can be used to build an equation that could predict success or failure for an embryo at the laboratory.
Trial registration number: N/A.

P-220 Morphodynamic observations at the 2-cell stage can be correlated to the occurence of direct cleavage from 2 to 5 cells

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Study question: Can fragmentation, multinucleation and symmetry at the 2-cell stage potentially be used to predict direct cleavage from 2 to 5 cells?
Summary answer: Multinucleation and asymmetry are higher at the 2-cell stage for embryos that undergo direct cleavage from 2 to 5 cells in less than 5 h.
What is known already: Evidence based data demonstrate that embryos directly cleaving from one to three cells have lower implantation rate than embryos with a normal cleavage pattern and thus detecting direct cleavage (DC) is important. DC of embryos from 2 to 5 cells is a less known phenomenon but also associated with less optimal outcome. Little is known about the morphological appearance of fragmentation, symmetry and multinucleation in these embryos and if these parameters may be used to predict DC 2to5 eventually even without the need of time-lapse equipment.
Study design, size, duration: This is a retrospective cohort study using data from all cycles that were incubated in a closed time-lapse system between July 2013 to October 2015. 1615 embryos were annotated for morphokinetic (2PN, tPNf, t2, t3, t4, t5, t8, tM, tSB, tB) and for morphological variables at the 2- and 4-cell stage (fragmentation, multinucleation, symmetry). The morphological variables, were examined at the middle of the 2-cell stage.
Participants/materials, setting, methods: For detecting direct cleaving embryos from two to five cells the formula t5–t3 was applied using 5 h as a cut-off value for subgroups that either showed direct cleavage (group one < 5 h) or not (group two 5 h). Embryos with direct cleavage 1 to 3 (t3–t2 < 5 h) were excluded from this analysis.
Main results and the role of chance: Embryos in group 1 with direct cleavage 2 to 5 showed later values for t2 (28.6 ± 4.0) and t3 (41.1±5.5) compared to group 2 (27.7±5.2; 39.5±5.9, respectively). Values for t8 did not differ between both groups. Fragmentation at the 2-cell stage was assessed at 31.2 h± 5.5 for group one and 31.6 h±6.7 for group two, multinucleation at 33.4 h±5.6 for group one, 33.5h ± 6.2 for group two and symmetry at 31.5 h±5.3 for group one, and 32.5 h± 8.3 for group two. These values represent the middle of the cell cycle of the 2-cell stage for respective groups. Degree of fragmentation did not differ between both groups. The incidence of multinucleation in 2- cell embryos was significantly higher (45.2%: 80/177) for group one compared to group two (30.3%: 436/1438) ($p < 0.0001$). Percentage of asymmetric embryos was also significantly higher in group one (72.5%: 161/222) compared to group two (53.6%: 849/1584) ($p < 0.0001$).
Limitations, reasons for caution: The timing of direct cleavage from two to five cell and the cut off values suggested should be controlled and adjusted for potential confounding parameters like patient's characteristics as well as changeable clinical and laboratory procedures.
Wider implications of the findings: Although the higher incidence of MN and asymmetry at the 2-cell stage may predict direct cleavage from 2- to5-cells, proper assessment can only be done in the middle of the cell cycle which predicts the use of time-lapse imaging. Implantation assessment of embryos in study groups is required.
Trial registration number: None.

P-221 Background signals and potential measurement errors in miRNA microarray analysis of IVF culture media

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Study question: What is the background signal when analyzing unconditioned culture medium with miRNA microarrays?
Summary answer: All of the 84 evaluated miRNAs were randomly detected in the samples with Ct values >30, most likely representing false positive calls.
What is known already: miRNAs are small single-stranded non-coding RNA molecules, serving as gene regulators at the post-transcriptional levels. miRNAs secreted into culture media from human IVF embryos have been suggested to correlate with implantation. However, the majority of miRNAs measured have been weakly expressed with Ct values above 30, which introduces suspicion of potential errors with false positive calls. Furthermore, previous studies using a limited number of samples have reported that miRNAs are present in unconditioned control medium in levels comparable to presumed secreted miRNAs from the embryos. A thorough evaluation of background signals is crucial before concluding on clinical significance.
Study design, size, duration: miRNA expression was evaluated in unconditioned culture media (media in which no embryos have been cultured). Three commercial media were evaluated: Sydney IVF Blastocyst medium, G2 protein-free (Vitrolife) and GTL (Vitrolife). Two different batches were evaluated from Sydney IVF Blastocyst medium and one batch from each of the other two. Four samples of 200 µl were analyzed from each batch (16 samples in total). RNAase free water was used as negative control.
Participants/materials, setting, methods: All procedures were performed according to the manufacturer's (Qiagen) protocol. *C. elegans* miR-39 was added as an internal spike-in control along with MS2 carrier RNA to optimize purification. miRNA was isolated using miRNeasy Serum/Plasma kit. cDNA was generated using the miScript II RT kit followed by 12 rounds of pre-amplification using miScript preAMP. 84 specific miRNAs expressed during cellular and organism development were quantified using the Human Cell Differentiation & Development miScript miRNA PCR Array.
Main results and the role of chance: The Ct values of the positive PCR control and the RT control were both within the recommended range (19±2 and C_T^{miRTC}-C_T^{PPC} < 2, respectively). There was no difference between the Ct values

(11–12) of the added spike-in miRNA, the positive PCR control and the RT control between the samples, which indicates low inter-assay variability. All of the 84 miRNA were randomly detected in one or more of the samples. With a few exceptions the Ct-values were above 30, which is the limit the manufacturer sets for negative calls. Similarly, the 6 normalizers suggested by the manufacturer (SNORD61/68/72/95/96A and RNU6B) were all detected in one or more media samples with Ct values comparable to the Ct values for the 84 specific miRNAs. For two of the samples the Ct value of one of the suggested normalizers (SNORD68) was 28.
Limitations, reasons for caution: For two of the media types, only samples from one batch was analysed. The Ct values may reflect the presence of contamination introduced during the workflow. As the 84 miRNAs detected were randomly distributed between the samples, we do not consider this explanation plausible. Only one platform (Qiagen) was tested.
Wider implications of the findings: This methodology study demonstrates that Ct values >30 obtained with the procedure employed most likely represent background signals or false positive calls, and should be interpreted with great caution. Using miRNAs expressed at extremely low levels as normalizers may introduce significant bias or result in false positive detection of miRNAs.
Trial registration number: NA.

P-222 Effect of ICSI with Calcium ionophore on embryo fertilization, multinucleation, direct cleavage and morphokinetics

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Study question: Does Calcium Ionophore (Ica) affect ICSI outcomes?
Summary answer: The use of ICa does not affect the fertilization, embryo multinucleation, pregnancy and implantation rates, direct cleavage proportion or the embryo morphokinetics, expect tPB2.
What is known already: Artificial oocyte activation (AOA) with ICa has shown successful ICSI outcomes in patients with previous fertilization failure. However, information regarding the impact of ICa on embryo development is scarce.
Study design, size, duration: Retrospective cohort study of 65 couples with severe male factor (sperm Samples under 1 mill/ml) were studied. 271 oocytes from 36 couples who underwent ICSI with Ica (Ca2+ group) were compared to 232 microinjected oocytes from 29 couples (control group), with severe male factor (SMF), between January 2011 and December 2015 in IVI VIGO Clinic.
Participants/materials, setting, methods: AOA was performed by injecting the oocytes with spermatozoa in a buffered media with Ica. Later, they were incubated for twenty minutes with culture media and ICa in a 37°C, 6%CO2 atmosphere. Embryo culture was carried out in a time lapse monitored incubator. Fertilization, embryo multinucleation at 2 and 4 cells, pregnancy, implantation rates and direct cleavage events and embryo morphokinetics, were analysed. χ^2, *t*-Student and Mann–Whitney tests were employed when applicable.
Main results and the role of chance: Fertilization rate was similar between Ica and control group, 54.2% vs. 58.1% ($p = 0.06$). No differences among groups were found for abnormal fertilization (1.3 or 4 pronucleai) rate. No differences in embryo multinucleation rate at 2 and 4 cells between Ica and control group were found; $p = 0.8$ and $p = 0.09$ respectively, and the proportion of direct cleavage events was not different ($p = 0.447$) either. Pregnancy rate was similar in the Ica group, 55.55% vs. 60.8%, as well as the implantation rate, although without statistically significant differences. Embryo morphokinetics tPB2, tPNa, tPNf, t2 to t8, cc2, and s2 were analyzed among groups and there were statistically significant differences on tPB2. We suppose that Ica may affect the calcium signals patterns that trigger embryo fertilization, and it could be reflected at the time at which the second polar body is extruded (tPB2)
Limitations, reasons for caution: The Ica embryos analyzed were obtained from patients with SMF with previous complete fertilization failure, and compared to embryos from SMF patients. The conclusions reached may not be applicable for patients with other ethyologies. Sample size is still limited and more cycles are needed to draw firm conclusions.
Wider implications of the findings: Our findings offer new data about the embryos behaviour under AOA with Ica, reflect the safety of its application.
Trial registration number: none.

P-223 Compaction dynamics of the morula stage embryo defined by time-lapse monitoring predicts blastocyst formation rates and quality

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Study question: Can compaction characteristics of day 4 morula stage embryo visualized by time-lapse systems be an objective marker to define blastocyst formation and quality?
Summary answer: Fully compacted embryos on day 4 have a significantly higher rate of blastocyst formation and good quality blastocysts compared to partially compacted day 4 embryos.
What is known already: Despite the fact that day 3 cleavage stage embryo morphology, cell number and kinetic characteristics had been correlated with blastocyst formation rate and quality, few studies examined the effect of day 4 morula stage embryo morphology on same parameters. Since compaction of mammalian embryos represents the first morphological event reflecting differentiation of the inner cell mass and trophoectoderm, the characteristics of compaction on day 4 could play an important role on the quality and viability of the resultant blastocyst.
Study design, size, duration: 113 patients with a mean age of 34.1 and without severe endometriosis or severe male factor were analysed retrospectively from February 2014 to December 2015. A total of 566 blastocysts obtained were monitored for 5 days in a time-lapse incubator. Change in morula morphology were continuously visualized from the time at which blastomere fused and compaction was completed until the time of the beginning of blastulation.
Participants/materials, setting, methods: A total of 100 blastocysts were of top quality with the score ≥4AA based on Gardner grading scheme. The characteristics of compaction were divided into two groups. First group of blastocysyts originated from fully compacted morulae whereas the second group developed from partially compacted morulae. The outcome parameters were blastocyst formation rates and number of ≥4AA blastocysts.
Main results and the role of chance: Blastocyst formation rate from fully compacted morulae in the first group was 97.0% (197/203) whereas it was 91.7% (369/402) from partially compacted morulae in the second group ($p < 0.01$). The incidence of ≥4AA blastocysts was 49.2% (100/203) for the first group and 7.4% (30/402) for the second group which was found to be highly significant ($p < 0.001$). Mean timing for the blastomere fusion and compaction visualized by time-lapse was 89.1 ± 0.58 h in the first group, and 92.6 ± 0.45 in the second group, whereas the start of blastulation in the first study group was 99.0 ± 0.54, and 102 ± 0.46 h. For the second group. The timing difference for the blastomere fusion and compaction as well as the blastulation start was significantly delayed in the second study group compared to the first one ($p < 0.0001$).
Limitations, reasons for caution: Patient related factors were not investigated in subgroup analysis as potential confounding parameters for blastocyst formation and quality despite compaction morphology. Despite the fact that same culture media was used during the entire study, it needs to be noticed that different culture media may influence the time of compaction.
Wider implications of the findings: Our findings underline the importance of day 4 morula stage embryo compaction characteristics on blastocyst formation rates as well as blastocyst quality. The implantation potential of such blastocysts should be further investigated to analyse whether the viability potential of these embryos are different in the study groups.
Trial registration number: None.

P-224 Correlation between oocyte yield, blastocyst formation and euploidy: longitudinal cohort study in advanced maternal age population

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Study question: Is metaphase II oocyte (MII) yield in an IVF/ICSI cycle correlated to blastocyst and euploid blastocyst (eblastocyst) formation rates per inseminated MII?
Summary answer: Ovarian response to stimulation is unrelated to oocyte quality in terms of blastocyst formation and chromosomal status in advanced maternal age (AMA) population.
What is known already: The ovarian response and thus the number of MII available for insemination is a function of the woman's ovarian reserve and type of ovarian stimulation protocol used. Low or excessive ovarian response, have previously been suggested to have a detrimental effect on oocyte quality. The aim of this study was to analyse the possible correlation between the size of oocyte cohorts retrieved (oocyte yield), oocyte development competence and chromosomal status in a single stimulation protocol group of AMA patients. Aneuploidy testing (PGD-A) was performed at blastocyst stage with comprehensive chromosomal analysis. Primary outcome measures were: Blastocyst/MII and eblastocyst/MII rates.
Study design, size, duration: Longitudinal cohort study preformed between April 2013 and December 2015. Only IVF/ICSI cycles with PGD-A were included. Severe male factor infertility was excluded. All patients underwent the same protocol for ovarian stimulation using FSH and LH in combination with GnRH antagonist starting on day 2 of the cycle. The dose was adjusted according to patients and cycle characteristics with the aim to maximize the number of oocytes to be retrieved.
Participants/materials, setting, methods: 847 patients undergoing 1046 cycles were included. Data are presented as mean+SD and percentages with 95%CI for continuous and categorical variables, respectively. Mean female age was 39.3+2.0 years (range 35–42). Overall, 7285 MII oocytes were obtained and inseminated by ICSI (6.9+4.5, range 1–25). Effects were estimated with multivariable analysis using mixed logistic regression models with a subject-specific intercept to take into account unobserved heterogeneity due to repeated measurements in the same woman.
Main results and the role of chance: 2532 blastocysts were obtained and analysed. Overall, 34.8% (range 26–100%, 95CI: 33.7–35.9) and 15.8% (range 20–100%, 95CI: 15.0–16.7) inseminated MII oocytes developed to blastocyst and to eblastocyst, respectively. Day of development to blastocyst stage/MII was 14.3% (95CI: 13.5–15.2), 17.7% (95CI: 16.8–18.6) and 2.7% (95CI: 2.4–3.1) for day 5, 6 and 7, respectively. Blastocyst/MII rate was not related to oocyte yield (OR: 0.990, 95%CI: 0.98–1.00, $p = 0.144$). Similarly, eblastocyst/MII rate was not associated to oocyte yield (OR: 0.995, 95%CI: 0.85–1.0, $p = 0.55$). The statistical analysis was adjusted for confounders including patient and cycle's characteristics. Only female was significantly related to outcomes. Blastocyst and eblastocyst/MII rates decreased significantly with increasing female age (OR: 0.930, 95%CI: 0.912–0.948, $p < 0.001$ and OR: 0.828, 95%CI: 0.809–0.980, $p < 0.001$, respectively). A relative decrease of 7.0% and 17.2% in the probability to reach blastocyst/MII and eblastocyst/MII respectively was observed for each additional year of age.
Limitations, reasons for caution: This study has been performed in a selected population of AMA patients treated with a single protocol for ovarian stimulation aimed at maximizing the oocyte yield. Results cannot be applicable to different population and protocols.
Wider implications of the findings: We show that ovarian reserve, measured as the response to a specific stimulation protocol, doesn't compromise oocyte competence. Poor ovarian response is thus only associated to a decline in oocyte quantity but not in quality.
Trial registration number: none.

P-225 The impact of severe endometriosis on embryo quality and pregnancy rate after intracytoplasmic sperm injection (ICSI)

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Study question: Study question: Do the presence of severe endometriosis (grade III–IV) leads to reduce embryo quality and pregnancy rate after ICSI?
Summary answer: Summary answer: Severe endometriosis decreases fertilisation and top quality embryos which imply with significantly decreasing in clinical pregnancy.
What is known already: No consensus concerning the impact of severe endometriosis on oocyte and embryo quality as well as pregnancy rate from women who underwent in vitro *fertilization* (IVF)/ICSI. Some studies have suggested that endometriosis reduced quality of oocytes and embryos and impaired uterine receptivity thus reduced pregnancy rate. In contrast, several studies have presented that endometriosis patients who underwent IVF/ICSI had comparable outcomes compared to those with tubal factors infertility.
Study design, size, duration: A case control study of patients ≤ 35 years old undergoing ICSI at BROS hospital between September 2010 and August 2015. 65 patients in control group were diagnosed with tubal factors infertility whilst 23 patients were diagnosed with severe endometriosis diagnosed by laparascopy according to American Fertility Society (AFS) classification (treatment group). Patients with other factors besides tubal factors infertility and severe endometriosis were excluded. Only patients with first cycle were included.
Participants/materials, setting, methods: Before undergone hormonal stimulation, treatment group was treated with Leuprorelin acetate and endometrioma cysts (diameter ≥3 cm) were taken out. All patients were administrated with rFSH (short protocol). Ovarian stimulation parameters were observed based on dose of rFSH, days of stimulation, number of follicles, oocytes and maturation. Variables in assessment of embryos included fertilisation, cleavage, and number of top quality embryos on day 3. Maximum 3 embryos were transferred. Clinical pregnancy was confirmed by heartbeat.
Main results and the role of chance: There were no significant differences in basal serum LH, FSH or estradiol and number of follicles between women with severe endometriosis and women with tubal factors infertility. Women with severe endometriosis required more days of ovarian stimulation compared to women with tubal factors infertility (8.2 ± 0.9 versus 7.6 ± 1.1, $p < 0.05$, respectively). There was a trend towards an increasing of dosage of stimulation ($p = 0.07$) but decreasing in number of oocytes ($p = 0.06$) and mature oocytes ($p = 0.06$) in treatment group compared to control group. Fertilisation rate and number of top quality embryos were significantly higher in control group compared to treatment group (5.3 ± 0.4 versus 3.8 ± 0.3, 3.1 ± 0.3 versus 2.2 ± 0.3, $p < 0.05$, respectively). These implied with significantly decreasing in clinical pregnancy of women with severe endometriosis (29.2%) compared to women with tubal factors infertility (55.4%, OR 3.0, 95% CI, 1.11 to 8.16). There is a higher pregnancy with two gestational sacs/pregnancy rate (14.3% versus 30.6%) and triple gestational sacs (0% versus 11.1%, respectively) in women with tubal factors infertility compared to those with severe endometriosis.
Limitations, reasons for caution: The excision of endometrioma cysts with diameter ≥3 cm before starting hormonal stimulation may reduce ovarian reserve in women with severe endometriosis.
Wider implications of the findings: While the findings are mostly in agreement with previous studies that severe endometriosis reduced embryos quality and pregnancy rate after ICSI, this present study also shows that pre-treatment endometriosis with Leuprorelin acetate and endometrioma excision do not improve outcomes of ICSI in the same level as women with tubal pathology.
Trial registration number: N/A.

P-226 Vitrification of oocytes and blastocysts using a fully automated cryopreservation device

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Study question:: Vitrification has become the standard technique for oocytes and embryo cryopreservation, but it is time consuming and operator-dependent. Can the procedure be fully automated?
Summary answer:: This is the first report of a fully automated vitrification device (called Sarah) enabling the standardization and simplification of the entire process.
What is known already: Currently vitrification is the method of choice for preserving oocytes and embryos. However, the procedure is cumbersome, requires highly skilled personal and is not yet standardized. Having an automated device allowing the exposure of oocytes or embryos to the various vitrification solutions, including the final immersion into liquid nitrogen (LN), will overcome all the above mentioned disadvantages.
Study design, size, duration: Experimental research using oocytes and embryos collected from the oviducts of 6 weeks old mice (CBA x BL C57) superovulated with PMSG and hCG and vitrified using a novel fully automated device (Sarah). Upon rewarming the oocytes and embryos were assessed for viability, cleavage, blastocyst and hatching rates.

Participants/materials, setting, methods: Oocytes (n = 20) or day 2 embryos (n = 60), with 40 (study group) automatically vitrified and 20 cultured fresh (controls). Up to 6 oocytes/embryos were first loaded into 1/4cc straws end-closed by special grids (50 µm pores) and then connected to the cryo-device having a computerized rotating cylinder containing cups with increasing concentrations of vitrification/equilibration solutions. After passing through the various stations, the straws were also automatically plunged into LN slush.
Main results and the role of chance: Upon rewarming, oocyte survival was evaluated by return into the isotonic volume while viability was assessed by live/dead staining. Rewarmed d2 embryos were placed in culture and their viability was evaluated by in vitro growth assessing blastocyst and hatching rates. Results showed that 95% (19/20) of the MII oocytes regained isotonic volumes and all (100%) of the surviving were viable according to the live/dead stains. Rewarmed embryos (study group) had 95% (38/40) blastulation rate (d4) and 75% (30/40) hatching rate (d5). The fresh embryos (controls) had a similar hatching rate of 80% (16/20, p NS).
Limitations, reasons for caution: This work was done only on mice embryos and oocytes and has not yet been performed on human oocytes or embryos.
Wider implications of the findings: Currently, there is no methodology able to fully automate the vitrification process. Here we reported a method that by virtue of a fully automated, operator-independent process, including the immersion into LN, will simplify and standardize the vitrification procedures worldwide.
Trial registration number: N/A.

P-227 Time Lapse analysis of the interrelationship between direct cleavage, multinucleation and maternal age in Natural Cycle IVF and Standard IVF

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Study question: Do Natural Cycle embryos display direct cleavage, and is multinucleation or maternal age related? Are there measurable differences between Natural Cycle and Standard IVF embryos?
Summary answer: No statistical relationship between direct cleavage, multinucleation and maternal age in Natural Cycle embryos. Statistically lower parameters, with improved embryo viability, compared with stimulated cycles.
What is known already: Direct cleavage and the presence of multinucleated cells are both associated with increased aneuploidy and reduced viability in developing embryos with low blastocyst formation and implantation. The stage at which direct cleavage occurs affects the embryo viability differently. When direct cleavage occurs in the 1st cell cycle (1–3 cells), the embryo viability is reduced dramatically to almost zero. Whereas, if it occurs in the 2nd or 3rd cycles (2–5 cells & 5–9 cells) then viability is reduced but euploid embryos are found after PGS and clinical pregnancies are generated.
Study design, size, duration: This is a retrospective study including 515 embryos from Natural Cycle IVF and 525 embryos from stimulated IVF cycles. Embryos were monitored in tri-gas incubator with time lapse (Embryoscope™, UniSense, Denmark). Data from cycles carried out between Jan 2013–Dec 2015. All Embryos developed up to 8 cell stage. Annotations were performed by the same person.
Participants/materials, setting, methods: Patients undergoing Natural Cycle IVF and Standard IVF were randomly allocated to time lapse incubation immediately after standard ICSI procedure. All normally fertilised oocytes were annotated by standard procedure for timings of cleavages, the presence of micronucleation (2 cell stage), and direct cleavage (any cell dividing into 3 distinct components with nuclear structures). Statistical analysis Chi-squared contingency test performed on graphpad software and t test for means.
Main results and the role of chance: Of 515 embryos generated from natural cycle, direct cleavage was observed in 23.7% of all embryos (11.3% in 1st cleavage, 11.8% in 2nd cleavage, & 0.6% in 3rd cleavage). Multinucleation (MN) was assessed in 390 embryos at 2 cell stage (42% 0 MN, 18% MN (1 cell), 40% MN (both cells). With advancing maternal age (<38 & >38 yrs) there were no significant differences with respect to direct cleave or multinucleation, and viability score for embryos also showed no significant difference with increase in maternal age (A+B score 48.1% vs. 53.2% and Excluded 29% vs. 22% for <38 yr vs. >38 yr groups). In contrast, there were significant differences between natural and stimulated cycles. Direct cleavages were lower in natural cycles 23.7% vs. 33.2% [natural vs. stimulated (p = 0.02)], and embryo viability score elevated in natural cycles [A+B classification (51.6% vs. 41.9%) natural vs. stimulated (p = 0.002)]. The presence of MNs were statistically different in both groups, but with increased levels in natural cycles 52.0% vs. 43.0% [natural vs. stimulated (p = 0.02)].
Limitations, reasons for caution: The numbers of elective natural cycle cases are minimal, the majority have history of previous poor stimulation outcome or advanced maternal age.
Wider implications of the findings: In depth analysis of embryos derived from natural cycle IVF allows us to uncouple developmental factors inherent to the oocyte/embryo from those induced/enhanced by ovarian hyperstimulation protocols. There is consensus that higher dosage in poor responders increases oocyte quantity at the expense of quality.
Trial registration number: Not applicable.

P-228 Effect of the well-of-the-well culture system on development of human embryos – a prospective randomized study with sibling embryos

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Study question: To evaluate the effectiveness of the well-of-the-well (WOW) culture system (LinKID® micro25, DNP, Japan) on development of human in vitro fertilization (IVF) treatments.
Summary answer: The WOW system improved blastocyst development, especially in good-quality cleavage stage embryos.
What is known already: The WOW dish contains micro-wells in the center of the dish, allowing for the identification of individual embryos cultured together in the same microdrop. It has been reported that the WOW system improved blastocyst development of bovine embryos.
Study design, size, duration: This prospective randomized study included 1,108 embryos from 109 IVF cycles between July and December 2015. The trial's sample size was determined by the primary trial endpoint of the blastocyst formation rate. Patients were enrolled when six or more normal fertilized oocytes (2PN) were achieved
Participants/materials, setting, methods: A sibling analysis was performed. The 2PN were randomly placed in two groups and allocated to either the WOW or individual drop culture (control group) for direct comparison. In the WOW group, three or more embryos were cultured together in 50 µL of culture media. The control group embryos were placed into a 25 µl droplet individually. Furthermore, we compared blastocyst development according to cleavage quality (good/poor) on Day 3 in both culture groups.
Main results and the role of chance: Good-quality cleavage rate on Day 3 was similar in both groups (67.6% in the WOW group vs. 67.0% in the control group). However, blastocyst development showed a significant difference; there were significantly higher blastocyst formation rate and usable embryo rate (available for either fresh transfer or cryopreservation) in the WOW group than in the control group (61.2% vs. 54.3%, and 60% vs. 53%, respectively; p <.05). In addition, poor-quality cleavage stage embryos showed no difference in blastocyst development regardless of the culture system (33.7% in the WOW group vs. 33.3% in the control group). In contrast, good-quality cleavage stage embryos in the WOW culture resulted in a higher blastocyst formation rate and usable embryo rate (73.8% vs. 64.2%, and 74.6% vs. 64.8%, respectively; p < .05).
Limitations, reasons for caution: Our results did not include pregnancy or live birth rates. Further studies including this data are required to clarify the link between embryo quality and the effect of the culture system.
Wider implications of the findings: Our results suggest that the WOW system may have a beneficial effect on embryo development. The system may improve culture conditions via autocrine factors from the embryos themselves, resulting in greater blastocyst formation.
Trial registration number: Not applicable.

P-229 Embryo Quality Assessment in in Vitro Fertilization (IVF) using metabolite footprints secreted to human embryo culture media by ATR-FTIR spectroscopy and multivariate analysis

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Study question: Can embryo quality be determined noninvasively using FTIR spectroscopy to analyze human embryo culture media in IVF treatment?
Summary answer: The embryos which provided pregnancy were diagnosed by FTIR spectroscopy with multivariate analysis with 100% sensitivity and specificity.
What is known already: The success level of IVF method is low with cumulative delivery rates of 47–51% per cycle. The greatest limitation of IVF method is not to know the quality of the embryo and thus unavailability to designate which embryo would result in pregnancy after transfer. Morphological assessment is the primary method used to determine embryo quality with the disadvantage of having a modest predictive value during IVF cycles. Therefore there is an urgent need for reliable methods to choose the most viable embryo for transfer and as a result of decreasing multiple gestations, as well as increasing overall pregnancy rates.
Study design, size, duration: In this prospective, double-blind study, embryo quality was determined by analysing spent culture media after fertilization by ATR-FTIR spectroscopy and hierarchical cluster analysis (HCA). For each patient, transferred embryos were selected according to morphological assessment and ATR-FTIR studies were carried out collaterally without knowing the morphological grading of each embryo. Therefore, this study also provides an accurate comparison of morphological assessment and ATR-FTIR results of the same embryos via culture media.
Participants/materials, setting, methods: 30 patients (24–36 years old) with unexplained infertility were included in the study. A total of 634 spent embryo culture medium samples were collected individually on days 3 and 5 for ATR-FTIR studies. A control medium sample incubated under the same conditions without an embryo was also collected as unspent controls. ATR-FTIR studies were carried out blinded to the morphological grading of the embryos, which embryo was transferred and which patient was clinically pregnant.
Main results and the role of chance: Among 30 patients, 10 patients got pregnant. Nine pregnancies gave birth after the IVF treatment, while one pregnancy was miscarriage. There was no success in transfer of morphologically selected embryo in the remaining 20 patients. In ATR-FTIR studies, the best results for the discrimination of spent culture medium samples of good quality embryos (embryos which provided pregnancy) were obtained with the cluster analysis performed on the vector-normalized spectra in the 4000–400 cm^{-1} wavenumber range. The embryos which provided pregnancy in 10 patients were diagnosed by clustering near the unspent culture medium (control) in the HCA dendrogram. Thus, best qualified embryos of these patients were diagnosed with 100% sensitivity and specificity (10/10) by FTIR spectroscopy. In 15 out of 20 patients with IVF failure, different embryos were diagnosed as best qualified in FTIR studies and morphological assessment. In the remaining 5 patients, although transferred embryos selected by morphological assessment were clustered as good-qualified embryos in FTIR studies, they could not provide pregnancy.
Limitations, reasons for caution: None.
Wider implications of the findings: Using FTIR spectroscopy to define embryo quality in IVF cycle may increase the success rates of pregnancy in IVF cycle by enabling the selection of the best qualified embryo. It may also contribute to prevention of multiple pregnancies by decreasing the transferred embryo number.
Trial registration number: None.

P-230 Effect of sperm DNA fragmentation on embryo quality; a morphology dynamics analysis

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Study question: Does sperm DNA fragmentation affect the morphokinetic parameters of embryo development?
Summary answer: Sperm DNA fragmentation delays the time to blastocyst stage but has no impact neither on the chance of suffering abnormal divisions, nor on embryo arrestment.
What is known already: Sperm DNA fragmentation (sDNAf) assessment has been introduced in many ART clinics as a diagnostic tool. Some authors find an increased miscarriage rate in patients with higher sDNAf, but the lack of a unique test to evaluate the damage and an agreed cut-off value make consensus difficult. Damaged DNA can be repaired by the oocyte and the embryo mechanisms, but it is still unclear if the time consumed by this phenomenon affects the embryo morphokinetics. Recent publications correlate irregular cell divisions with poor implantation potential, but we still don't know if they could be related to sDNAf.
Study design, size, duration: Retrospective, cohort study. This study includes 971 embryos from 135 ICSI cycles performed between March and December 2015.
Participants/materials, setting, methods: University-affiliated infertility clinic. Ejaculated samples from patients were processed through density gradients and sDNAf was analyzed by TUNEL assay after the oocytes were microinjected. Cycles were performed using oocytes from patients (56; 41.48%) or donors (79; 58.52%), which were fresh (71; 52.59%) or vitrified (64; 47.41%). Embryos were cultured in an EmbryoScope® incubator. Time of every cell cleavage and irregular division such as direct cleavage, incomplete cleavage, reverse cleavage and asynchronous cleavage were annotated and correlated with sDNAf.
Main results and the role of chance: Mean sDNAf observed in the neat samples was 14.30 ± 10.52%. The origin of oocytes and whether they were fresh or vitrified did not affect results. Length of cell cycles and cell synchrony were unaffected by the percentage of sDNAf, but embryos reached the blastocyst stage later when were obtained from samples with higher sDNAf ($p = 0.028$). The percentage of sDNAf was similar in embryos that blastulated compared to the ones that did not (13.80 ± 10.27% vs. 14.65 ± 11.12%). Normal cleavage was observed in 740 embryos (76.21%); direct cleavage from 1 to 3 blastomeres in 104 embryos (10.71%); direct cleavage from 1 to 4 blastomeres in 11 embryos (1.13%); incomplete cleavage in 44 embryos (4.53%); reverse cleavage in 42 embryos (4.32%) and asynchronous cleavage in 29 embryos (2.99%). Percentage of sDNAf was independent of the cleavage pattern, being 14.26 ± 10.52; 16.28 ± 11.65; 14.54 ± 13.93; 12.50 ± 8.82; 12.64 ± 9.55 and 13.64 ± 8.75, respectively.
Limitations, reasons for caution: The main limitation of this study is that female factor cannot be avoided, although we observed that results were independent of the origin of the oocytes and whether they were fresh or vitrified.
Wider implications of the findings: According to our results, embryos that come from samples with higher sDNAf present a delayed blastulation. In consequence we demonstrated how sDNAf affects embryo development and should be taken into consideration as a diagnostic tool of sperm quality. Reference values for sDNAf remain to be elucidated.
Trial registration number: Not applicable.

P-231 The impact of ovarian stimulation protocol on embryo morphokinetic parameters

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Study question: Which morphokinetic parameters are affected by the ovarian stimulation protocol?
Summary answer: Three morphokinetic parameters – s2, tM and SSBM showed significant difference, according to the ovarian stimulation protocol.
What is known already: The use of morphokinetic variables of human embryos as potential predictors of IVF outcome is becoming a promising tool for embryo selection in recent ART procedures. Many authors discuss the crucial importance of specific parameters, such as synchrony of the second cell cycle (s2), morula formation time (tM) and blastocyst formation time (tB). However, little is known about the effect of different concomitant factors (BMI, age, basal FSH levels and stimulation protocol) that may affect the early stages of embryo development and can subsequently influence the success of the applied ICSI or IVF procedures.
Study design, size, duration: This retrospective study was conducted from January 2012 to February 2015. A total of 420 embryos from 286 patients were studied.
Participants/materials, setting, methods: Patients were classified into two groups according to the ovarian stimulation protocols: (1) long protocol

(n = 138) and (2) short protocol (n = 148). The two groups were selected to be comparable and did not differ significantly with regard to age, BMI, or basal FSH levels. Embryos were cultured in a one-step medium and analyzed in Embryoscope™. 36 morphokinetic variables were analyzed. All statistical analyses were performed in SPSS-21 software.

Main results and the role of chance: Three of thirty six morphokinetic variables showed statistically significant difference ($p < 0.05$) between the studied groups of patients with long and short stimulation protocol, respectively: (1) Synchrony of the second cell cycle (s2), (2) morula formation time (tM) and (3) the period of time between the beginning of blastocyst formation and full blastocyst stage (SSBM = tSB − tM). The mean time-points in hours for the patients with long and short stimulation protocol were: 2.57 vs. 2.04 (s2), 99.82 vs. 87.80 (tM) and 10.92 vs. 15.00 (SSBM).

Limitations, reasons for caution: Data were collected from one laboratory and for this reason should not be assumed to be representative and applicable to all IVF laboratories before external validation. Choosing the specific culture medium, time-lapse system, control and monitoring software could lead to different time points and target morphokinetic parameters in each laboratory.

Wider implications of the findings: Better understanding of the direction and intensity of the impact of environmental factors on embryo development, such as patient treatment procedures would give an opportunity to improve the embryo selection, implantation outcome and to increase the live birth rate.

Trial registration number: NA.

P-232 Age dependent gene expression profiles of human ICSI blastocysts

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Study question: What is are the effective factors on the gene expression profiles of human intracytoplasmic sperm injection (ICSI) blastocysts ?

Summary answer: Maternal age had a larger effect on the gene expression profiles of single human ICSI blastocysts than the other considered factors (e.g., sperm motility rate).

What is known already: For humans, numerous studies indicate that detecting chromosomal abnormalities in embryos has beneficial effects on both the clinical implantation rate and the abortion rate. However, the relations between the human embryos' transcriptome profiles and the implantation rate and the live birth rate are unknown. Studies on mouse embryos have shown that the conditions of in vitro cultures and ICSI procedures can lead to alteration of the gene expression profiles. For humans, the joint effect of metaphase II (MII) oocytes and age has been demonstrated, but the joint effect of human blastocysts and age has been rarely considered.

Study design, size, duration: We analyzed 22 high quality day 5–6 cryopreserved embryos donated from patients who provided informed consent from three assisted reproduction centers in Japan between 2011 and 2015. The fertilization method only involved the ICSI procedure using fresh ejaculated spermatozoa.

Participants/materials, setting, methods: Ribonucleic acid (RNA) extraction from a single blastocyst was performed using the Arcturus PicoPure isolation kit. Gene expression profiles were analyzed with RNA sequencing using T7 RNA polymerase linear amplification. Statistical analyses of the gene expression data were performed with R software. We used Spearman's rank correlation coefficient to test for any association between age and the gene expression profiles.

Main results and the role of chance: We investigated 16 vitrified blastocysts from day 5, and 6 from day 6. The mean maternal age was 35.3 (31–43 years) and the mean paternal age was 37.6 (32–49 years). In the principal component analysis, the first and second principal components were correlated more strongly with maternal age and paternal age than with either the sperm motility rate, the speed of embryo development, embryo gender, or the timing of cryopreservation. The number of differentially expressed genes of maternal age is 990, and that of paternal age is 485. In most of those genes, maternal age and gene expression show a certain degree of inverse correlation. Gene ontology analysis by DAVID bioinformatics disclosed that maternal age dependent genes were involved in the eicosanoid metabolic process ($p < 0.01$), chromosome organization ($p < 0.01$), and nucleosome assembly ($p = 0.01$). Paternal age dependent genes were involved in amine transport ($p < 0.01$), T cell activation ($p < 0.01$), and negative regulation of proteolysis ($p < 0.01$). Maternal age dependent genes contain Aurora Kinase A (AURKA) ($p = 0.03$), Aurora Kinase C (AURKC) ($p < 0.01$), Pituitary Tumor-Transforming 1 (PTTG1) ($p < 0.01$), and Pituitary Tumor-Transforming 2 (PTTG2) ($p < 0.01$), which play crucial roles in cell cycle regulation, and therefore these genes have a higher possibility of human aneuploidy.

Limitations, reasons for caution: The link between gene expression and aneuploidy has not been adequately investigated. Furthermore, the freezing/thawing and the ICSI procedure might have caused changes to the gene expression profiles. Because of the small amount of materials, reverse transcription polymerase chain reaction (RT-PCR) was not performed.

Wider implications of the findings: We detected age-dependent gene expression profiles. Furthermore, when only euploid embryos were transferred, the implantation rate improved up to approximately 50–70%. Moreover, we believe that the combination of transcriptome assessment and preimplantation genetic screening (PGS) may potentially increase the implantation rate and live birth rate in the future.

Trial registration number: The study was approved by the Institutional Review Board of Tokyo Medical and Dental University in 2011 (IRB reference number: 2011-27-3-3).

P-233 Development and validation of a new Next Generation Sequencing-based Protocol to distinguish between balanced translocation and normal chromosomes embryos from robertsonian translocation carriers

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Study question: Can a new next generation sequencing (NGS)-based protocol be used for distinguishing between balanced translocation and normal chromosomes embros from robertsonian translocation carriers?

Summary answer: The results of this retrospective study demonstrate that this new protocol is accurate and reliable, allowing identification of balanced translocation embryos from robertsonian translocation carriers.

What is known already: Robertsonian translocation carriers do not normally have phenotypic manifestation, but lead to increased risk of infertility, miscarriage and live birth of chromosomally unbalanced offsprings. Preimplantation genetic screen (PGS) is an effective way to improve pregnant outcome for those couples. The detection of embryonic aneuploidy abnormality by PGS has been widely used in clinical. However, it can't distinguish between balanced translocation and normal chromosomes in embryos from robertsonian translocation carriers yet. Now we developed a new protocol based on NGS platform to resolve this problem and designed a retrospective study to evaluate it's accuracy and reliability.

Study design, size, duration: The study consisted of a retrospective study involving a double blind parallel evaluation, with our new NGS-based protocol, STR-based haplotype analyses and fetal karyotype validation, of 49 blastocysts obtained from 4 robertsonian translocation familes during the period of January to October 2015. Consistency of results from the NGS-based protocol was compared with the results obtained by short tanderm repeat markers (STR)-based haplotype analysis or fetal karyotype from prenatal diagnosis.

Participants/materials, setting, methods: Four robertsonian translocation familes undergoing comprehensive aneuploidy screening by NGS-based PGS were enrolled in the study. Written informed consent was obtained and the study was approved by the Ethics Committee. A high depth NGS on the region near the centromere of chromosomes involving robertsonian translocation is adopted

to build the haplotype of these robertsonian translocation chromosomes. Finally, according to the haplotype, we can accurately distinguish between normal embryos, balanced translocation embryos and non-equilibrium translocation embryos.

Main results and the role of chance: Forty nine blastocysts from four robertsonian translocation carriers' couples were successfully detected aneuploidies by comprehensive chromosomes screening using low depth NGS, 17 (34.7%) of which carried a copy number imbalance. Our high depth NGS in targeted Robertersonian chromosomes region was also successfully applied to all these embryos. Then we constructed the haplotype of these robertsonian translocation chromosomes according to the above NGS results. Among the other 32 euploid embros, 18 (56.3%) carried the derivative robertsonian chromosome haplotype and 14 (43.7%) carried normal robertsonian chromosome haplotype. These results were exactly the same as STR markers based haplotype analysis and fetal karyotype.

Limitations, reasons for caution: This protocol is not capable of applying for the familes which lack of abnormal robertsonian chromosomes' embryos. Although this result validate the new protocol can accurately identify the normal chromosomes embryos from robertsonian translocation carriers' embros, broad-based clinical data are need to define the role of the new protocol.

Wider implications of the findings: This is the first report, to our knowledge, of successful clinical application of targeted NGS-based protocol to distinguish between balanced and normal chromosomes embryos from robertsonian translocation carriers' couples.

Trial registration number: Not applicable.

P-234 Is allowing Metaphase I oocytes time to progress to metaphase II a waste of time? A cohort analysis of 9,632 oocytes (25)

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Study question: What is the developmental potential of Metaphase I oocytes and are the timings of oocyte pick-up (OPU) post-trigger, denudation and intracytoplasmic sperm injection (ICSI) important?

Summary answer: Late maturing oocytes have reasonable potential to produce euploid embryos and prolonging time-to-ICSI is associated with higher probability of first polar body extrusion and fertilization.

What is known already: Oocytes observed to be at metaphase I (MI) following denudation are thought to be severely compromised due to their lack of nuclear maturity. In vitro culture permits oocytes to continue their development, extrude the first polar body and progress to metaphase II (MII) hence allowing for ICSI to be performed. As a group, these oocytes, are believed to result in poor outcome potentially due to increased incidence of embryo aneuploidy. However, there are documented live births from oocytes originally assessed as immature following OPU. Currently, the exact developmental potential and the optimal management of these oocytes has not been established.

Study design, size, duration: This is a retrospective cohort analysis of 9,632 oocytes originating from all day-3 PGD/S cycles between 2011 and 2014. The exact time between the administration of the trigger for final oocyte maturation and the OPU (range: 33.7–40.4 h), between OPU and denudation (range: 0.2–6.2 h) and that between denudation and ICSI (range: 0.2–6.2 h) was recorded and extracted for each case. Furthermore, the nuclear maturation was assessed after denudation and extracted for the purposes of this study.

Participants/materials, setting, methods: Out of 9,632 oocytes collected, 1,225 were MIs (12.7%, 95% CI: 12.1–13.4) and at the time of ICSI 690 MIs had progressed to MIIs (56.3%, 95% CI: 53.5–59.1). These were followed up and fertilization rates, development of a good-or-top quality day-3 embryo and ploidy were assessed. Multivariable logistic regression analyses controlling for multiple confounders were performed with the use of the GEE framework in order to account for the non-independent nature of data.

Main results and the role of chance: As expected, the probability of MI-to-MII progression was increased with every additional hour between denudation and ICSI (odds ratio-OR: 1.72, 95% CI: 1.49–2.00), while it was reduced for every additional hour between OPU and denudation (OR: 0.43, 95% CI: 0.34–0.56) even after controlling for female age, type of protocol, type of trigger and time from trigger-to-OPU. Interestingly, the probability of fertilization was also higher in cases where the time between denudation and ICSI was longer (OR: 1.40, 95% CI: 1.06–1.83) after controlling for female age, type of protocol, type of trigger and time from trigger-to-OPU.

Trigger-to-OPU, OPU to denudation or denudation to ICSI was not associated with the probability of a good-or-top quality day-3 embryo formation or the presence of a euploid day-3 embryo after controlling for female age, type of protocol, type of trigger and time from trigger-to-OPU.

Out of all MI-to-MII oocytes injected, 5.3% (95% CI: 3.3–7.4) formed day-3 euploid embryos which was lower (OR: 0.39, 95% CI: 0.27–0.56) as compared to the proportion of MIIs that formed day-3 euploid embryos (12.6%; 95% CI: 11.5–13.6).

Ongoing pregnancy rates following a transfer of a single euploid blastocyst were not significantly different between embryos originating from MI-to-MII oocytes (6/24) and MII oocytes (172/487) (rate difference:-10.3%, 95% CI: −24.0 to +10.0%).

Limitations, reasons for caution: These patients have various indications requiring PGD/S and may not reflect the general subfertile population. Furthermore, solid conclusions regarding ongoing pregnancy rates cannot be drawn due to the small number of embryo transfers from the MI-to-MII cohort of embryos.

Wider implications of the findings: Delaying the timing of ICSI for MI oocytes seems to allow them to progress to MII oocytes and also seems to increase fertilization rates. These embryos, although less likely to be euploid compared to those originating from MII oocytes, can still add to the reproductive potential of an ICSI cycle.

Trial registration number: None.

P-235 Single Day 3 Embryo Transfer (D3SET) versus Single Blastocyst Transfer (SBLT) in young patients

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Study question: Comparison of the pregnancy rate in single embryo transfer on day 3 (D3SET) with single blastocyst transfer (SBLT) in young patients in terms of pregnancy rate.

Summary answer: D3SET pregnancy rate compared to SBLT was comparable. Significantly higher number of frozen embryos was achieved in D3SET but cumulative pregnancy rate should be examined.

What is known already: The trend of blastocysts transfer on Day 5 rather than day 3 embryos transfer has been recently more in use, since results seem to be better. Chocrane review on this topic showed live birth of 29.4% with Day 2 or3 ET compared with 36.0% in BLT. The cumulative pregnancy rate is even more complicated to follow because of the long period of time which these studies demand. In terms of multiples and missed abortions no differences were found between transfer of D2/D3 embryos and blastcysts.

Study design, size, duration: A prospective study of D3SET versus D5 SBLT was started 2 years ago. All patients at the age of ≤ 30 years were offered a single embryo transfer. After giving them the information of the possibility that no blastocyst development can lead to no transfer they could choose between D3SET and SBLT. Patients were allocated to this study only if it was their first cycle and had at least two top quality embryos on day3.

Participants/materials, setting, methods: The patients, who fulfilled the inclusion criteria, had to choose between D3SET and SBLT. Those who decided for D3SET, underwent the transfer, the remaining top quality embryos were vitrified. The intermediate qualified embryos were cultured to the BL stage and were frozen once they reached blastulation with Gardner Score (3–5AA, 3–5AB, 3–5BA). In the SBLT group the remaining blastocysts qualified with the same Gardner Score were also vitrified.

Main results and the role of chance: Twenty five patients at the age of 27.7 ± 4.27 y were in the D3SET group and 16 patients aged 25.6 ± 4.35 y in the SBLT one. Mean number of injected oocytes was 14.1 ± 7.8 compared with 18.0 ± 6.9. Fertilization and cleavage rates were comparable 10.7 ± 6.12, 14.2 ± 6.20 and 10.5 ± 6.23, 13.8 ± 6.32), respectively.

In the D3SET group; out of 263 embryos, 25 were transferred, 121 were frozen (46.0%), 44 of 109 cultured embryos, developed into BL (40.4%) of them 22 BLs were frozen (50%).

In the SBLT group; out of 221 embryos, 123 developed into BLs (58.3), 94 D5 AND 29 D6 (76.4% and 23.6% respectively). 16 BLs were transferred and 41 BLs (33%) were frozen. Altogether 143 (54.4%) embryos and BLs were frozen in the D3SET group and 41 (33%) BLs were frozen in the SBLT group ($p < 0.0001$). Eleven (44%) clinical pregnancies were achieved in the D3SET group, and 8 (50%) in the SBLT group (NS).

Limitations, reasons for caution: Since the study is based on SET, the time needed to get well established data is very long. Moreover, to examine the cumulative pregnancy rate in such groups in which the pregnancy rate are so high needs many years of follow up

Wider implications of the findings: In view of the results, the advantage of SBLT has still to be examined. The fact that pregnancy rate in these groups with such inclusion criteria demonstrated comparable results with significantly more frozen embryos on D3SET put in doubt the advantage of BLT in young patients

Trial registration number: NA.

P-236 Establishment of appropriate methods for human oocyte-activation by PLCZ1 cRNA (PLCZ) injection

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Study question: Is PLCZ injection into human oocyte a better method for oocyte-activation compared to other methods like Electrical stimulation or Ionomycin?

Summary answer: We found the optimal concentrations of PLCZ to activate oocytes physiologically, with a similar pattern of Ca^{2+} oscillations to that of in vitro fertilization oocytes.

What is known already: At fertilization, mammalian oocytes show repetitive transient Ca^{2+} transients each of which is due to Ca^{2+} release from the endoplasmic reticulum through Inositol 1,4,5-trisphosphate (IP_3) receptors. During fertilization, the so called sperm factor, is released into the oocyte and induces series of Ca^{2+} spikes that are required for oocyte activation. They are called Ca^{2+} oscillations. IP_3-producing enzyme phospholipase C zeta (PLCZ1) is a strong candidate to be the sperm factor.

Study design, size, duration: We performed this retrospective study to investigate the usefulness of PLCZ injection into human oocyte. 94 fresh M-II oocytes from 34 patients were used to find optimal concentration to activate human oocytes and 67 oocytes from 29 patients were used to compare with the other oocyte activation methods from January 2013 to December 2015.

Participants/materials, setting, methods: Optimal RNA concentration for oocyte activation was examined by injection with various concentrations of PLCZ into human oocytes from IVF patients who had consented to this experiments. After injection, the rate of PN formation was checked and the intracellular Ca^{2+} concentration of injected oocytes was monitored by Fluo8H AM fluorescent Ca^{2+} indicator.

Main results and the role of chance: Optimal concentration of PLCZ1 to activate human oocytes was 100 ng/μl in the examination using 94 fresh M-II oocytes (Table 1).

	Concentration of PLCZ (ng/μl)	0.01 (*n*=13)	0.05 (*n*=14)	0.1 (*n*=4)	1 (*n*=6)	5 (*n*=25)	10 (*n*=11)	100 (*n*=18)	1000 (*n*=3)
Day 1	Second polar body (%)	23.1	14.3	0	0	16.0	9.1	66.7	66.7
1PN (%)	23.1	14.3	0	0	8.0	9.1	66.7	66.7	

The pattern of Ca^{2+} oscillations by PLCZ injection was similar to the pattern of Ca^{2+} oscillations seen in the in vitro fertilized oocytes (Table 2).

	Oocytes (*n*)	Day 1	Day3 (≥7 cell)
1PN+ First polar body number (%)	Cleaved embryos number (%)		
Ionomycin	15	66.7 (10/15)	40.0 (4/10)
PLCZ1	35	65.7 (23/35)	60.1 (14/23)
Electrical stimulation	17	52.9 (9/17)	44.4 (4/9)

In comparison with the other oocyte activation methods, cleavage rate was the highest in the oocytes activated by PLCZ. However it showed no significant differences in pregnancy rates.

Limitations, reasons for caution: Birth of healthy offspring and reproduction of healthy second generation from mouse oocytes activated by PLCZ was reported in 2008. However healthy human births have not been reported after using this method. The safety of PLCZ injection to human health should be further examined for future clinical applications.

Wider implications of the findings: First it will rescue the unfertilized oocytes after ICSI due to insufficient oocyte activation, which accounts for approximately 40% of all unfertilized oocytes. Secondly, this new oocyte activating method which is more physiologically oocyte activation will be beneficial for improving clinical outcome of ROSI.

Trial registration number: None.

P-237 The moment of early cleavage in embryos originating from donor eggs, captured by time-lapse, determines morphological quality on days 2, 3 and 5

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Study question: The study's purpose was to establish a relationship between the moment of early cleavage and morphological embryo quality on days 2, 3 and 5.

Summary answer: Embryos that divided between 23.5–25.5 hours have a greater probability of developing good morphological quality on days 2, 3 and 5.

What is known already: Early cleavage can be used as an embryo selection factor. It is a morphokinetic marker directly linked to embryo morphology, implantation potential and pregnancy rate. The evolution of assisted reproduction has permitted the establishment of new non-invasive embryo selection methods such as time-lapse analysis systems (EmbryoScope®). The markers that this technology has helped define have displaced the first mitotic division as a non-invasive indicator of good embryo quality to using other indicators such as t3 and t5, which correspond to the appearance of the third and the fifth blastomere.

Study design, size, duration: Between November 2013 and March 2015, embryos originating from 879 donor eggs retrieved from 104 donors were retrospectively observed using a time-lapse system: EmbryoScope®. Out of the total, 481 embryos were expanded blastocysts on day 5. The moment of early cleavage of these embryos was analyzed in relation to their morphological quality. The ASEBIR (Spanish Association of Reproduction Biology) classification was used for day 2 and 3 embryos. For day 5, Gardner's classification was utilized.

Participants/materials, setting, methods: This study took place in a private fertility clinic in Madrid, Spain. Embryos originating from 879 donor eggs were included in this analysis. Exclusion criteria were cryopreserved ovocytes, severe masculine factor and PGD. Embryos were classified according to the moment of the first cellular division and their morphological quality on days 2, 3 and 5. The time of early cleavage was analyzed based on sensitivity, specificity, predictive value, probability coefficient and efficiency.

Main results and the role of chance: According to our results, embryos that divide between 23 and 26 hours present good morphological quality on days 2 and 3. In the analysis of blastocysts, better morphological quality is found in those embryos with early division between 24 and 25 hours (Specificity; 0.86, Sensitivity 0.17). In studying the relation between embryo development on days 2, 3 and achieving the blastocyst stage, our data show better embryo quality in those whose first division took place between 24 and 25 hours (Specificity = 0.90 Sensitivity = 0.22). Moreover, we observed that those embryos with inferior quality presented early division before 23 hours on day 2 and 3 and after 25.5 hours when analyzing the intervals on day 5.

Limitations, reasons for caution: Our study only evaluated healthy women between 19 and 29. Due the fact that not all transfers were single embryo transfers pregnancy rates were not reported.

Wider implications of the findings: Because early cleavage is a morphokinetic feature, its exact time can be observed almost exclusively using time-lapse systems. The optimum time for cleavage to take place has been defined in this study. It is an easy, non-invasive prediction marker of embryo quality on days 2, 3 and 5.

Trial registration number: None.

P-238 Is pregnancy rate following transfer of high quality blastocysts vitrified at day 6 comparable to that of blastocysts vitrified at day 5?

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Study question: Is there a difference in pregnancy rates between high quality blastocysts vitrified on day 6 versus blastocysts vitrified on day 5 after fertilization?
Summary answer: Transferring blastocysts vitrified on day 5 gave a higher clinical pregnancy rate compared to high quality blastocysts vitrified on day 6.
What is known already: The implantation rate and clinical pregnancy rate is higher after transferring blastocysts compared to cleavage stage embryos, during fresh and frozen cycles. Previous studies have showed decreased pregnancy rate for transferring blastocysts day 6 compared to blastocysts day 5 during fresh cycles. However, studies involving frozen thawed blastocyst transfers have reported conflicting results regarding whether the rate of blastocyst formation prior to cryopreservation affects treatment outcome.
Study design, size, duration: This was a retrospective cohort study of 820 freeze/thaw cycles of blastocysts vitrified either on day 5 or day 6 and transferred between January 2012 and October 2015. Vitrification in these cases was carried out on day 6 due to insufficient expansion of the embryos on day 5.
Participants/materials, setting, methods: 820 vitrification warming cycles and embryo transfers were included in the study. 555 of the cycles included blastocysts vitrified at day 5 and 265 cycles included blastocysts vitrified at day 6. The embryos were warmed and, transferred on day 6 of progesterone in hormonally prepared cycles.
Main results and the role of chance: The age of the patients and the proportion of embryos that survived the warming process were comparable between the two groups. More top quality embryos were transferred in the group in which blastocysts were vitrified day 6 (1 vs. 1.1, $p < 0.001$) but the clinical pregnancy rate (32% vs. 44%, $p = 0.002$) and the ongoing pregnancy rate (28% vs. 41%, $p < 0.001$) were higher in the group in which blastocysts were vitrified on day 5. Multivariate regression analysis adjusting for patient's age, number of embryos transferred, number of top quality embryos transferred (≥3BB) and treatment protocol demonstrated that the day 6 vitrified group had a significantly lower clinical pregnancy rate compared to the day 5 vitrified group (OR 0.54, 95% CI 0.38–0.76).
Limitations, reasons for caution: There are other factors that can influence the pregnancy rate such as the physician or the embryologist performing the transfer, difficulty inserting the transfer catheter, endometrial thickness and patter, subendometrial contractions to name a few. Those factors were not controlled for in the study.
Wider implications of the findings: Although the day 6 blastocyst morphology after warming is at least as good as that of blastocysts vitrified on day 5, the clinical pregnancy rate following frozen embryo transfer is significantly lower with blastocysts vitrified on day 6 compared to blastocysts vitrified on day 5.
Trial registration number: none.

P-239 A comparative analysis of fertilization rate and clinical outcome in sibling human oocytes fertilized by split insemination in patients with teratozoospermia

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Study question: What are the laboratory and clinical outcomes of intracytoplasmic sperm injection (ICSI) vs. conventional insemination using sibling oocytes in IVF cycles for patients with teratozoospermia?
Summary answer: The fertilization-rate and blastocyst quantity are statistically higher with ICSI than with conventional IVF. Sub-analysis showed this was due to those with strict morphology ≤ 4%.
What is known already: Kruger proposed that sperm classification on the basis of strict morphologic criteria is a predictor for fertilization. However, this has fallen out of favor with some studies having shown fertilization rates are unaffected by morphology. Few studies have evaluated ICSI and spontaneous sperm penetration at IVF cycles in sibling oocytes.
Study design, size, duration: All patients treated by a physician with Kruger morphology ≤ 9% on semen analysis from 01-2013 to 12-2015, had 50% ICSI, 50% IVF ordered, for their first IVF cycle. Up until 2013 all patients with morphology ≤ 5% had 100% ICSI ordered. Felling evidence no longer supported this, but fearing fertilization failure, 50% ICSI was ordered instead. Subsequently permission was obtained to evaluate the database retrospectively. Patients with orders were recorded prospectively. 140 had IVF ordered.
Participants/materials, setting, methods: 124 patients underwent IVF, 103 patients underwent 50% ICSI, 50% IVF and were included in the study. 20 patients had 100% ICSI due to poor sperm quality as determined by the laboratory at the day of insemination. One patient had 100% IVF performed due to immature oocytes.

Paired samples statistics were used for laboratory outcome comparisons. Chi squared tests were used for pregnancy outcomes. Data is presented as mean ± standard deviation.
Main results and the role of chance: A total of 1162 mature oocytes were obtained: 606 were assigned to IVF and 556 to ICSI by the embryologists in a blinded fashion. The mean number of oocytes per patient was 14.2 ± 8.6, mean MII oocytes were 5.9 ± 4.2 and 5.4 ± 3.8 in the conventional IVF and ICSI groups respectively ($p = 0.04$), favoring IVF.

The fertilization rate in ICSI was higher than in conventional IVF (74.28% vs. 47.02%, $p < 0.0001$). 100% fertilization failure was reported in 25 patients' IVF and 6 patients' in ICSI ($p < 0.0001$).

The mean number of day 2 embryos was higher in ICSI as compared to IVF (3.95 ± 3.26 vs. 2.76 ± 2.93, $p = 0.001$) and similarly day 3 embryos ($p < 0.0001$). There were more blastocysts from ICSI that with IVF ($p = 0.006$) and they were better grade ($p = 0.049$).

Sub-analysis showed that ICSI was statistically favored over IVF only in cases with morphology ≤ 4. Comparison of fertilization rates, embryo quantity and quality lost significance when evaluate couples with morphology ≥5.

Best embryo for transfer was selected blinded by the embryologists. No difference occurred between ICSI and IVF for: pregnancy rates ($p = 0.189$), clinical pregnancy rates ($p = 0.487$), miscarriage rates ($p = 0.465$), and live birth rates ($p = 0.44$).
Limitations, reasons for caution: The study's main limitations are the relative small sample size and its retrospective design. Nevertheless, this drawback seems to be outweighed by the fact that medical records included complete clinical information, and no patients were lost since they were prospectively enrolled in the database.
Wider implications of the findings: ICSI should be used when normal sperm morphology ≤ 4%. ICSI improves fertilization and embryo quality and quantity in this group. Conclusions cannot be drawn from pregnancy outcomes due to the bias created by not randomizing choice with intention to treat, which fails to account for no fertilization in IVF.
Trial registration number: No trial registration.

P-240 Group vs. individual culture performed in single medium: a prospective randomized sibling-oocyte study

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Study question: Does group culture perform better than individual culture using single medium in term of blastocyst formation rate (BFR) and overall blastocyst quality?
Summary answer: Group culture does not show any improvement compared to individual culture in term of BFR and overall blastocyst quality when performed in single culture medium.
What is known already: Individual culture offers an easy way to follow embryo development individually, however, different authors have demonstrated that group culture improves embryo viability in mice, cattle, felids and mammals. These studies have been conducted using different media, mainly belonging to the category of sequential ones. To our knowledge no studies applying a prospective comparison between individual and group culture have been conducted in human IVF using single medium without refreshment and leaving embryos undisturbed from day-0 up to the blastocyst stage.
Study design, size, duration: This was a prospective randomized study of 163 sibling oocytes conducted from September to December 2015. Injected oocytes were randomly assigned to Group A and Group B and allocated individually or

in groups of 3–4 oocytes respectively in dish wells. Randomization was performed by splitting the oocytes under a stereomicroscope not allowing for identification of oocyte quality.
Participants/materials, setting, methods: A total of 21 women (aged ≤ 39 years) undergoing oocyte retrieval procedure for intracytoplasmic sperm injection (ICSI) at Ferticlinic, Villa Margherita, were involved in the study. Culture was performed without refreshment in 35 µl of single-step medium (One-Step, SAGE) up to blastocyst stage in multi-gas incubators (Sanyo) at 37°C, 5% 02, 5.5% CO2. Blastocyst formation rate was the main outcome measure.
Main results and the role of chance: No statistically significant difference was detected in the fertilization rate between group A and B, 74.6% vs. 77.1% respectively, ($p = 0.755$). No statistically significant difference was detected on day-3 with regard to cleavage rate, 100% vs. 96.4% ± 12.0 ($p = 0.186$) and top quality embryo rate, 70.6 ± 36.8% vs. 69.2% ± 28.9 ($p = 0.853$) for group A and group B respectively. On day-5, no significant difference was detected between the two groups with respect to BFR (group A 69.7% ± 31.3 vs. group B 66.3% ± 9.9, 95% CI = −11.3–18, $p = 0.638$) and top-quality blastocyst rate (group A 35.2% ± 42.1 vs. group B 30.2% ± 42.6, $p = 0.686$).
Limitations, reasons for caution: This was a prospective randomized study with a limited sample size. The number of patients should be increased in order to confirm these preliminary data and clinical outcomes should be examined as well.
Wider implications of the findings: We speculate that individual culture in single medium probably recreates a sort of "group effect": in group-culture the beneficial effect is due to paracrine interactions that enhance autocrine factors, while in single-culture it is determined by the accumulation of autocrine factors thanks to the use of single medium without refreshment.
Trial registration number: None.

P-241 Cell exclusion during embryo development: is there an incidence? Analyse of more than 1500 time-lapse recording

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Study question: The study aim to evaluate the impact of cell exclusion during compaction on blastocyst formation and implantation using the Embryoscope®, a time lapse monitoring system.
Summary answer: Cell exclusion impacts blastocyst formation and the proportion of good quality blastocyst obtained but it does not show an effect on implantation rate.
What is known already: The onset of compaction can occur from the 4-cell to the 16-cell stage embryo follows the activation of the embryonic genome. At this stage, blastomeres begin to compact tightly and cell boundaries progressively disappear until the fully compaction, this, having an important influence on the subsequent processes of blastocyst formation.
Study design, size, duration: We retrospectively analysed all the embryos obtained after ICSI cycles cultured up to the blastocyst stage in the Embryoscope® over a 3 years period in our IVF unit. Embryos were classified according to morphological aspect at the compaction stage, i.e., with or without cells out of the morula. 1552 videos of embryo development were analysed with 570 embryos in the cell exclusion group and 982 in the control group.
Participants/materials, setting, methods: Immediately after ICSI, oocytes were placed into the Embryoscope®. Each embryo was investigated by detailed time-lapse analysis measuring the exact timing of the developmental events in hours after ICSI procedure. We particularly observed the time of compaction and the number of cells involved in it in order to see any cell exclusion.
Main results and the role of chance: No difference was found in embryos characteristics of both groups in kinetic development until the onset of compaction, where embryos showing excluded cells reached compaction later than the control group: 101.9 ± 11.9 h vs. 95.9 ± 10.5 h to reach compaction, $p < 0.01$. Moreover, we also noted a significantly higher proportion of abnormal cleavage (1 to 3 or 2 to 5 cells) in cell exclusion group compared to the control group (24.2% vs. 10.2%, $p < 0.01$) In addition, we observed significantly fewer good quality blastocysts (i.e., eligible for transfer or cryopreservation) in the cell exclusion group than in the control group (33.8% vs. 58.5%, $p < 0.01$). Nevertheless, regarding single embryo transfer, no difference was found in terms of pregnancy rate (41.7% vs. 48.7%) or on on-going pregnancy (31.6% vs. 33.6%).
Limitations, reasons for caution: This study was retrospective and monocentric, preventing from generalizing our results to other ART centres.
Wider implications of the findings: Time-lapse systems are known to optimize embryo culture thanks to uninterrupted culture and continuous surveillance. In this study we reported that although cell exclusion seems to delay compaction and impact blastocyst formation, it has, no effect on the result implantation rate.
Trial registration number: none.

P-242 PI3K signaling Pathway gene expression analysis in cumulus cells by Real-Time PCR

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Study question: Could be cumulus cells gene expression profile explored as potential markers of oocyte competence?
Summary answer: Data have shown selective modulation of transcripts involved in the PI3K signaling pathway in relation to IVF outcome.
What is known already: CCs closely interact with the oocyte and share the same microenvironment. CCs gene expression analysis provides an indication of the microenvironment in which oocyte maturation undergoes. DNA microarray technology has been used to analyze the CCs transcriptome obtained from ART patients receiving different stimulation protocols. Differential gene expression has been taken as evidence of modifications in signaling and metabolic pathways that could potentially affect oocyte quality. In particular we recently evidenced the expression modulation of the AKT/PI3K pathway, important mediator of cumulus-oocyte complex development.
Study design, size, duration: To understand if the expression of CCs genes in the PI3K pathway could be potential markers of oocyte competence we analyzed the expression of 92 transcripts of this pathway in 10 CCs from patients with negative IVF outcome and 10 CCs associated to positive outcome. Samples inclusion criteria: derived from cumulus-oocyte complexes containing mature oocytes showing polar body and associated to single embryo transfer. The negative/positive outcome has been respectively defined by implantation failure/success.
Participants/materials, setting, methods: Cumulus-oocyte complexes were collected at the University of Pisa and Hospital of Catania IVF laboratories from 20 healthy patients undergoing standard IVF protocols. Total RNA was extracted from CCs using the Arcturus Pico Pure RNA Isolation Kit. A Real Time approach was used to analyze PI3K pathwaygene expression loading a 96-well custom TaqMan Array. Expression data of CCs associated with positive IVF outcome were compared to data from negative outcome samples.
Main results and the role of chance: Data analysis showed the selective modulation of transcripts involved in the PI3K Signaling Pathway in relation to IVF outcome. In particular our analysis demonstrated that 11 transcripts in this pathway were significantly down-regulated in all samples of positive CCs when compared to negative CCs. This expression pattern seems to modulate mainly the apoptotic processes in CCs. Among these genes, AKT1, BCL2L1, and SHC1are particularly interesting; they, in fact, are known to regulate CCs growth and apoptosis. These data could indicate that CCs associated to positive outcome oocytes show specifically a reduced expression of genes involved in apoptosis regulation, whereas higher expression of the same transcripts in CCs associated to negative outcome, could reflect oocyte with low rate of maturation and quality.
Limitations, reasons for caution: High sensitivity of CCs transcriptome to intrinsic and extrinsic factors related to the patient, such as metabolic status, reproductive health and hormonal stimulation.
Wider implications of the findings: The selected non-invasive biomarkers could represent prognostic factors to assess oocyte competence in IVF patients

to increase live birth rate. Moreover, a final extensive analysis of the specific role of the modulated transcripts could be useful to unravel the molecular basis linking oocyte quality and developmental CCs.
Trial registration number: NA.

P-243 Embryo development after continuous culture in two commercial single-step media: a prospective randomized study with sibling oocytes

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Study question: Is embryo development different after continuous culture in Sage 1-step vs. Continuous Single Culture (CSC) media?
Summary answer: Embryo development is similar after continuous culture in Sage 1-step or CSC media.
What is known already: The formulation of single-step media has allowed the culture of preimplantation human embryos to the blastocyst stage in an uninterrupted and continuous manner without media renewal. Several single media have recently become commercially available, and therefore comparison of their efficacy is justified.
Study design, size, duration: Prospective randomized study including 633 oocytes retrieved from 42 IVF patients between October 2014 and April 2015. Randomization software was used to allocate oocytes from each cohort between groups in a 1:1 ratio.
Participants/materials, setting, methods: A total of 633 metaphase-II oocytes from 42 women (aged ≤ 40 years, with ^{3}10 oocytes retrieved) were randomly allocated to continuous culture in either Sage 1-step medium (Origio) ($n = 316$) or CSC medium (Irvine Scientific) ($n = 317$). Embryo transfers were performed on Day 3 (9 patients) or Day 5 (31 patients). Two patients had all blastocysts frozen on Day 5 due to occurrence of severe OHSS. Values are expressed as mean (95% CI).
Main results and the role of chance: Fertilization rates [65.8% (58.4–73.2) vs. 67.1% (60.5–73.7), $p = 0.749$] and cleavage rates [95.1% (92.2–98.0) vs. 98.3% (96.3–100), $p = 0.076$] were similar in Sage 1-step and CSC groups. On Day 3, proportion of embryos with good morphology [62.5% (52.4–72.6) vs. 64.3% (54.9–73.7), $p = 684$], proportion of embryos with ≥6-cells [81.5% (74.0–89.0) vs. 83.3% (75.9–90.7), $p = 0.734$] and proportion of embryos with ≤ 10% fragmentation [68.8% (58.6–79.1) vs. 68.6% (59.2–78.0), $p = 0.975$] did not differ. In the subgroup of 33 patients who had embryo culture/transfer on Day 5, blastocyst formation rates [53.6% (45.0–62.2) vs. 51.9 (42.6–61.2), $p = 0.755$], proportion of at least full blastocysts [45.0% (36.5–53.5) vs. 44.0% (35.5–52.50, $p = 0.850$], proportion of good quality blastocysts (≥3BB) [36.8% (28.5–45.1) vs. 36.1% (27.5–44.7), $p = 0.897$], and proportion of blastocysts with grade A inner cell mass [64.6% (52.7–76.5) vs. 56.7% (43.6–69.8), $p = 0.372$] were comparable in Sage 1-step and CSC media, respectively. The proportion of blastocysts with grade A trophectoderm [67.2% (56.9–77.5) vs. 48.7% (36.4–61.0)] appeared to be higher in Sage 1-step medium compared to CSC medium ($p = 0.037$). The proportion of embryos transferred [26.1% (20.4–31.9) vs. 21.0% (14.6–27.4)], and frozen [28.8% (21.0–36.6) vs. 33.8% (24.5–43.1)], as well as embryo utilization rates [55.6% (47.7–63.5) vs. 54.8% (45.7–63.9)] were similar in Sage 1-step and CSC media.
Limitations, reasons for caution: This is a prospective randomized study with sibling oocytes comparing embryo characteristics in Sage 1-step and CSC media. In order to study the comparative effect of these two single-step media on the probability of pregnancy it is necessary to perform an RCT in which the randomization unit will be patients.
Wider implications of the findings: Continuous culture of embryos in Sage 1-step and CSC media is associated with similar embryo development and utilization. Both single-step media appear to provide adequate support during in-vitro preimplantation embryo development.
Trial registration number: NCT02302638.

P-244 A prospective randomized controlled study (RCT) depicting better blastocyst outcome and pregnancy rate after single step media culture vs sequential media culture in IVF-ICSI Cycles

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Study question: This study was to encourage a minimum of embryo manipulation and to determine if sequential media are really necessary in order to meet the changing requirements of embryo?
Summary answer: Study shows Better blastocyst outcome after use of single-step-media, allowing the embryo itself to choose the necessary nutrients while maintaining a more stable culture environment.
What is known already: It has been suggested that sequential-media that mimic the physiological conditions in the female reproductive tract are required for optimal development of the human embryo to the blastocyst stage. Sequential-media were introduced in order to meet the changing requirements of the developing embryo in vitro. However, there has been renewed interest in the use of single-step-media, which allow the embryo itself to choose the necessary nutrients while maintaining a more stable culture environment. Previous studies suggest that sequential media do not appear superior to single-step media. Use of culture media that encourage a minimum of embryo manipulation must be explored.
Study design, size, duration: To compare blastulation and pregnancy rates in two different embryo culture systems: single step Versus Sequential culture from day 1 to day 5, 184 patients below 40 years of age were included between 2014 and 2015, who were randomised into two groups by a computer generated list, after IVF-ICSI. The same protocol was applied. Primary outcome measured were number of Blastocysts obtain at Day 5 and kinetics of blastocyst. Secondary outcome were pregnancy rates. Participants/materials, setting, methods: 84 infertile patients below 40 years of age were included between 2014 and 2015, were randomised into two groups by a computer, after IVF-ICSI cycles. In random half of the oocytes were treated with a sequential system the other half with a single step. All embryos are cultured by group under oil overlay. Primary outcome measured were number of Blastocysts obtain at Day 5. Secondary outcome measured were implantation and pregnancy rates.
Main results and the role of chance: The blastulation rate obtained with the Single-Step-Media are 43% vs. 40% with the Sequential-Media. Pregnancy rates with the Single-Step-Media are 39% vs. 37% with the Sequential-Media. Results also show faster kinetics with more bastocytes with the single steps culture but clinical pregnancy rate were not significantly different in the two groups ($p > 0.05$). (Student *t*-test and chi square test). The objective of this study was to determine if sequential media are really necessary.
Limitations, reasons for caution: This study shows an effect of culture-Media for human preimplantation embryos on embryo quality and success-rates. The existing data are insufficient to allow the selection of the best culture medium and thus more designed RCTs are necessary for both currently used culture media as well as newly introduced culture media.
Wider implications of the findings: Results show more blastocysts available for transfer and vitrification with the use of Single-Step-Media although clinical pregnancy rate were not significantly different in the two groups. The continuous uninterrupted protocol, without medium renewal remains in effect without detriment to clinical outcomes. Less manipulation of embryos remains a priority in order to reduce epigenetic risks.
Trial registration number: BTTBC/2014/09.

P-245 Predictive value of human calcium pattern analysis on the benefit of assisted oocyte activation in cases of failed fertilization after ICSI

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Study question: Does human calcium pattern analysis contribute to predict the benefit of assisted oocyte activation (AOA) after ICSI-failed fertilization?

Summary answer: Human Oocyte Calcium Analysis (HOCA) predicts AOA outcome. HOCA is more sensitive than heterologous-ICSI tests to reveal sperm-related oocyte-activation deficiencies in sperm causing failed-fertilization after ICSI.

What is known already: Heterologous ICSI-based tests such as mouse oocyte activation test (MOAT) and mouse oocyte calcium analysis (MOCA) are used to discern whether ICSI-failed fertilization is due to sperm or oocyte factors. The diagnosis is valuable to counsel AOA in following cycles. Although they can reveal severe sperm deficiencies, presumably related to the oocyte-activation factor PLCζ, AOA doesn't benefit all cases in which sperm activation capacity was considered normal. Human-PLCζ shows greater potency than mouse-PLCζ and certain sperm defects could remain undetected due to this variability. Thus, human sperm capable of activating mouse oocytes, may still fail to induce human oocyte activation.

Study design, size, duration: Patients included showed fertilization failure after ICSI and moderate (70–84%) to normal (>85%) oocyte activation rate after MOAT. As is practiced in our setting, ionophore based-AOA was applied to 50% or 100% of the oocytes depending on the MOAT result. All patients were further analysed by MOCA and HOCA to determine the calcium oscillations profile compared to sperm samples with known fertilization potential as control. The results were retrospectively contrasted with the AOA outcome.

Participants/materials, setting, methods: Donated oocytes discarded from IVF/ICSI, as oocytes containing *smooth endoplasmic reticulum aggregates (SER)* and GV or MI in vitro matured MII were collected and vitrified. After ICSI, Ca^{+2}-oscillations were detected using a radiometric method for a duration of 2 h (MOCA) or 10 h (HOCA) using the Ca^{+2}-indicators Fura2/P3E-AM (λ_{exc}340/380 nm), respectively. Product of amplitude (ΔFluorescence from baseline, A) and frequency (total number oscillations, F), and area under the curve (total calcium released, AUC) were calculated using Clampfit_10.2.

Main results and the role of chance: Patients studied (n = 10) showed moderate (n = 3) to normal activation rates (n = 7) after MOAT. For MOCA, AxF >9 was established as normality threshold by previous studies. According to this, AxF was <9 in 4 cases (AxF_{mean} = 6.38 ± 2.60 a.u), including the 3 patients with moderated MOAT. For HOCA, control sperm showed the following results: n oocytes = 20; AxF = 6.77 a.u. and AUC = 5.10 a.u. Interestingly, 6 patients showed a significant reduced sperm activation capacity compared to the control (n oocytes = 7.83 ± 1.17; AxF_{mean} = 0.07 ± 0.13 a.u. and AUC 0.08 ± 0.13 a.u.), including the 4 cases with reduced MOCA score. The other 4 patients showed similar sperm activation capacity compared to the control (n oocytes = 9.25 ± 0.96; AxF_{mean} =6.78 ± 1.25 a.u. and AUC = 7.82 ± 1.44 a.u.). Clinical data showed that AOA-ICSI had a significant benefit in all 6 patients with a reduced sperm activation potential, resulting in a mean fertilization rate of 79.5 ± 17.64%; n oocytes/ICSI = 9.83 ± 4.06 after ICSI-AOA. In the 4 patients with normal HOCA score the application of AOA-ICSI did not restore fertilization rates (7.58 ± 15.15%; n oocytes/ICSI = 14.50 ± 12.34).

Limitations, reasons for caution: *IVM* conditions might impair oocyte quality, however, their use to study calcium dynamics after fertilization has been already validated. Although the use of SER-oocytes can lead to healthy babies, their clinical use is still a concern due to certain reported malformations and genetic abnormalities.

Wider implications of the findings: Our data support the added-value of calcium analysis, especially using human oocytes, to uncover sperm-related activation deficiencies in cases of failed fertilization after ICSI. HOCA indicates whether AOA would be beneficial in a following cycle. When failed fertilization occurs and the Ca^{2+}-analysis shows normal patterns, oocyte deficiency could be suspected.

Trial registration number: n/a.

P-246 Karyokinesis without cytokinesis in human embryo – A time-lapse study

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Study question: To investigate the prevalence of karyokinesis without cytokinesis (NUK) in early cleavage stage human embryos and their developmental competence and implantation potential.

Summary answer: The incidence of NUK in the first three cleavage stages ranged from 0.26% to 5.09%. Blastocyst development and implantation rates were impaired.

What is known already: Bovine embryos exposed to a protein kinase inhibitor exhibited karyokinesis without cytokinesis during the first mitotic cell cycle, resulting in polyploid blastocysts. This phenomenon was also observed in human embryos resulting in the formation of multinucleated blastomeres, which are associated with higher aneuploid rates and lower developmental potential.

Study design, size, duration: A retrospective cohort study of IVF patients between January and June 2015. A total of 6,464 normally fertilized embryos from 783 cycles (674 ICSI and 109 IVF) were included in the study All embryos were cultured in time-lapse incubator.

Participants/materials, setting, methods: Embryos were cultured in time-lapse incubators (EmbryoScope®, Vitrolife, Sweden). One or more blastomeres exhibiting karyokinesis without cytokinesis (nuclear division only – NUK) were identified. These embryos were classified into NUK-1 (at first cleavage), NUK-2 (at second cleavage), NUK-3 (at third cleavage) or NUKPlus (occurring at more than once). Blastocyst rate and clinical outcome were analyzed using Chi-square test.

Main results and the role of chance: The NUK incidence in the early embryo cleavage stages were 0.26% (at first), 2.90% (at second), 5.09% (at third) and 0.58% (at fourth), with 1.61% of the embryos having two or more episodes (NUKPlus). Overall NUK incidence was not significantly different between IVF and ICSI (10.80% vs. 10.25%, p = 0.60) or between oocyte age groups (9.12%–12.52%, p = 0.27). The good blastocyst formation rate was significantly lower in NUK embryos (35.29%) compared to embryos without NUKS (64.80%, p < 0.001). Fetal heart implantation rates, based on known implantation data (KID), were lower in NUK groups (D3-ET 5.88%; D5-ET 20.00%) compared to NUK-free groups (D3-ET 15.10%; D5-ET 37.50%). Due to the smaller KID sample size (D3 n = 17, D5 n = 5), they were not reaching significance (D3 p = 0.29 D5 p = 0.41).

Limitations, reasons for caution: The non-significance for implantation data is due to the small KID sample size and multiple embryos transferred.

Wider implications of the findings: Embryos demonstrating karyokinesis without cytokinesis should be exclude from embryo transfer selection and may improve the success rate in SET.

Trial registration number: None.

P-247 Low lactate culture benefits the in vitro development of preimplantation mammalian embryos

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Study question: Is lower lactate in the culture media beneficial to in vitro embryo development?

Summary answer: Continuous culture in medium containing a low lactate concentration benefits the quality of in vitro mammalian embryo development when compared to higher concentrations of lactate**.**

What is known already: Preimplantation mammalian embryos primarily metabolize pyruvate during the cleavage stage, when glucose utilization is relatively low. Glucose metabolism increases steadily during compaction and becomes the predominant metabolite at the blastocyst stage, with increased production of lactate. The interaction of lactate and pyruvate utilization at different stages of development appears to play a role in the regulation of pyruvate and glucose metabolism during embryo development. Current lactate levels used in human embryo continuous culture media were developed based on physiological levels determined from analysis of mouse tubal fluid, which differs from lactate levels determined in human tubal fluid.

Study design, size, duration: Mouse (1-cell) or bovine (2-cell) embryos were cultured to blastocysts (day 4 or day 7, respectively) in continuous single culture medium containing HSA (5 mg/mL) with varied concentrations of lactate. Seven experiments were conducted for each animal model. The criterion for validity of the Mouse Embryo Assay (MEA) is ≥80% blastocyst rate and for Bovine Embryo Assay (BEA) is ≥30% blastocyst rate. Spent media were collected and analyzed for lactate, pyruvate and glucose levels.

Participants/materials, setting, methods: Mouse embryos (1-cell fresh or frozen) were pooled and randomly allocated (n = 25–30/medium) into low and high lactate media (3–4 embryos/20 µl) under oil. In vitro produced fresh

bovine 2-cell embryos were pooled and randomly divided (n = 30–40) among media conditions (10 embryos/50 µL) under oil. Blastocyst development rates were graded by morphology on day 4 for mouse embryos and day 7 for bovine embryos. Kits (Bioassay Systems) were used for lactate, pyruvate and glucose analysis.

Main results and the role of chance: In seven experiments with MEA the mean blastocyst rate was 92.0%, with 83.6% hatching rate for low lactate and 87.3%, with 62.0% hatching rate for high lactate. In seven BEA experiments the mean blastocyst rate was 52.4% for low lactate and 38.6% for high lactate. In addition, the grade of bovine blastocysts based on morphology was superior in low lactate than in high lactate culture. In all tests overall, the blastocyst rates for mouse and bovine experiments were higher for the low lactate than the high lactate media. These results suggest lower lactate in culture benefits overall rates of blastocyst development, and hatching in particular. Analysis of lactate production and consumption of pyruvate and glucose in spent embryo culture media collected from a subset of experiments further demonstrated different metabolic profiles of embryo resulting from media containing varied lactate concentrations.

Limitations, reasons for caution: These results suggest that lower lactate concentrations can benefit in vitro embryo development in both mouse and bovine embryo model system. Additional studies are required to determine if these findings are statistically significant, and how such benefits may translate to human embryo culture in vitro.

Wider implications of the findings: The apparent benefit to embryo development of low lactate medium represents a targeted approach to help elucidate how preimplantation embryo metabolism may be influenced by the culture environment, and how culture conditions may support and promote utilization of Warburg effect mechanisms to improve in vitro embryo development and viability.

Trial registration number: NA.

P-248 Zinc chelation promotes oocyte activation in human oocytes

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Study question: Does Zinc (Zn^{+2}) depletion play a role during oocyte activation and meiotic resumption in human oocytes?

Summary answer: Zn^{+2}-depletion induces successful oocyte activation bypassing Ca^{+2} mobilization in human oocytes. TPEN (N,N,N′,N′-tetrakis (2-pyridylmethyl)ethane-1,2-diamine) is presented as a potential activation agent for assisted oocyte activation strategies.

What is known already: The Zn^2-ion has been identified within oocyte cytoplasm in different mammalian species, including human. Intracytoplasmic Zn^{2+} increases during oocyte maturation and upon fertilization, a drastic Zn^{+2}-efflux has been described as a gatekeeper-event for meiosis resumption occurring downstream of the Ca^{+2}-oscillations. MII oocytes treated with TPEN, a specific Zn^{+2}-chelator, promoted meiotic resumption and parthenogenetic development that in turn resulted in full-term development in murine and porcine models. The presence of Zn^{+2}-sparks during human oocyte activation has been described, but the question remains whether artificial Zn^{+2}-depletion could be used as an assisted oocyte activation strategy to overcome failed fertilization after ICSI.

Study design, size, duration: The effect of TPEN on oocyte activation was first validated using mouse oocytes. Two-cell formation was evaluated 20 h after activation and compared to a control group ($SrCl_2$-10 mM). Human oocytes were exposed to TPEN and ionomycin (control). PN-formation and cleavage rates were registered at 20 h and 44 h after activation. The absence of Ca^{+2}-oscillations was confirmed using a radiometric method using Fura2-AM (λ_{exc}340/380 nm) as the Ca^{+2}-indicator during TPEN exposure (1 h) in mouse and human oocytes.

Participants/materials, setting, methods: Mouse oocytes (B6D2F1) were exposed to TPEN (100 µM; 45 min). After washing, test and control groups were cultured in media containing Cytochalasin-D (2 µg/ml) for a duration of 6 h. Both groups were transferred to KSOM-BSA (4%). Donated human oocytes containing *smooth endoplasmic-reticulum aggregates (SER),* were collected and vitrified. After thawing, oocytes were cultured for 2 h before activation. Oocytes were then exposed to TPEN (100 µM; 45 min) and ionomycin (10 µM; 2 × 10 min, control) and cultured in *Cook-Cleavage*. PN-formation and cleavage rates were evaluated.

Main results and the role of chance: Mouse oocytes exposed to TPEN and $SrCl_2$ showed similar activation rate: 92% (12/13) and 93% (14/15), respectively. In human oocytes, TPEN (100 µM) induced parthenogenetic activation in 54% (7/13) of the oocytes whereas ionomycin activated 75% (6/8) of the oocytes in the control. All the oocytes activated showed 2 to 4 cells at D+2. Cleavage rate was 57% (4/7) and 83% (5/6), for TPEN and control groups, respectively. To investigate whether TPEN induces artificial activation bypassing the calcium oscillations, the calcium profile was determined in mouse and human oocytes during their exposure to the activation agents: $SrCl_2$, ionomycin, and TPEN. No oscillations were observed after exposure of mouse and human oocytes to TPEN for a duration of 45 min. Control mouse oocytes exposed to $SrCl_2$ showed normal calcium activity for a duration of 2 h. Ionomycin induced a large peak of calcium in human oocytes.

Limitations, reasons for caution: Although the use of SER-oocytes can lead to healthy babies, their clinical use is still a concern due to certain reported genetic abnormalities. The influence of different concentrations of TPEN on activation and morphokinetics needs to be further analysed.

Wider implications of the findings: Current AOA protocols promote intracytoplasmic calcium rise to induce the oocyte activation. Our results show that artificial depletion of Zn^{+2} can successfully activate human oocytes by circumventing the event of calcium oscillations. It remains to be investigated whether this novel strategy could be optimized for AOA to overcome ICSI-fertilization failures.

Trial registration number: n/a.

P-249 Blastocyst morphology correlates differently with ploidy in relation to maternal age

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Study question: Does blastocyst morphology correlate with ploidy? If yes, how? Is there any difference between parameters involved in relation to maternal age?

Summary answer: Blastocysts with lower morphological scores were aneuploid with higher frequency. Aneuploidy correlated with trophectoderm quality in older patients and with expansion degree in younger ones.

What is known already: Aneuploidy is one of the most important causes of implantation failure and miscarriage. The association between morphological appearance and aneuploidy risk has been already studied with the aim to assist in identifying the most competent embryos to transfer during standard IVF procedures. Morphology seems to correlate with ploidy. At the blastocyst stage, aneuploidies were found to be significantly more frequent among embryos with poor morphological scores except for those able to achieve clinical pregnancies. Aneuploidy seemed to correlate with poorer trophectoderm scores also. Up today no correlation between blastocyst morphology and ploidy was studied in relation to patient's age.

Study design, size, duration: Relationship between age, morphology and ploidy was assessed retrospectively on arrayCGH data of 287 blastocysts subdivided in 4 groups: 1 (excellent), 2 (good), 3 (average) and 4 (poor) quality. Secondly, blastocysts were divided in 2 groups based on maternal age: 83 from women ≤ 35 years old (A) and 204 from women >35 years old (B). Expansion, inner cell mass and trophectoderm quality scores were assessed individually in groups A and B and associated with ploidy.

Participants/materials, setting, methods: Blastocyst morphology was assessed on day 5/6 according to Gardner and Schoolcraft grading system modified by Cornell University and trophectoderm biopsy was carried out. Array comparative genomic hybridization (aCGH) was performed for Comprehensive Chromosome Screening (CCS) for biopsied samples. Logistic regression analysis was used to assess the relationship between variables of blastocyst morphology and aneuploidy.

Main results and the role of chance: Blastocysts with lower morphological scores showed a stronger link to aneuploidy (group 3: p = 0.005, OR = 3.370,

95% CI 1.453–7.818; group 4: $p = 0.02$, OR = 2.815 95% CI 1.156–6.854). Maternal age was significantly associated with aneuploidy ($p = 0.0001$, OR = 1.187 95% CI 1.109–1.270) infact, aneuploidy rate was statistically different among group A and B (19.6% vs. 80.4%, $p = 0.0001$). Analyzing separately each single morphological blastocyst parameter (expansions, inner cell mass and trophectoderm), expansion degree was statistically associated to ploidy in Group A (mean age = 32.3, 95% CI 31.4–33.2): more expanded blastocysts were less aneuploidy (expansion 3, $p = 0.016$; OR = O.2; 95% CI 0.054–0.745) with euploid/aneuplod ratio of 1:0.2; on the contrary in less expanded blastocysts (expansion 1), euploid/aneuploid ratio was 1:1.3. In Group B (mean age = 40.3, 95% CI 39.7–41) logistic regression analysis showed that only trophectoderm quality was predictive of ploidy: worst quality trophectoderm was associated to aneuploidy (TE Grade B: $p = 0.001$, OR = 3.6 95% CI 1.746–7.664; TE Grade C: $p = 0.028$, OR = 2.5 95% CI 1.103–5.765).

Limitations, reasons for caution: Limited number of cases of the younger study group.

Wider implications of the findings: In the vast majority of IVF laboratories of the world the choice of the most suitable embryos to transfer is still based upon morphological evaluation. The findings of this CCS study can provide additional information helpful in creating a more accurate methodology for blastocyst grading when PGS is not used.

Trial registration number: none.

P-250 Comparing blastocyst expansion dynamics between euploid vs. aneuploid embryos: A quantitative and automated analysis of time-lapse cinematography

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Study question: What are expansion dynamics for human blastocysts cultured in vitro? Is there an association between blastocyst expansion patterns and embryonic aneuploidy?

Summary answer: In embryos biopsied on Day5/6, 89% expanded >10% on Day5. Euploid blastocysts had a higher degree and faster rate of expansion than aneuploid blastocysts.

What is known already: Blastocyst collapses have been found to associate with a lower likelihood to hatch and/or implant in animal and human studies. However, quantitative characterization of blastocyst expansion, including the degree and rate of expansion, has not been systematically performed. Furthermore, the relationship between blastocyst expansion dynamics and aneuploidy remains unknown.

Study design, size, duration: Prospective observational cohort study. One hundred and two patients were enrolled (August 2012 to November 2013), out of which seventy patients did not have laser-assisted hatching performed on Day 3 and were included for analysis. Sixty-six patients with 374 biopsied blastocysts had complete image analysis and ploidy outcome ($n = 258$ euploid and $n = 116$ aneuploid).

Participants/materials, setting, methods: Entire cohorts of embryos from patients were imaged using the Eeva System (111 hours post ICSI). Time-lapse images were automatically processed by proprietary computer programmes to extract dynamic parameters including the degree and rates of single expansion events. Forty embryos were excluded as they did not reach 10% expansion criteria on Day 5 ($n = 25$ euploid and $n = 15$ aneuploid), and the final dataset comprises of 334 embryos that expanded more than 10% ($n = 233$ euploid and $n = 101$ aneuploid).

Main results and the role of chance: Blastocysts spent a median of 86% time in expansion phase vs. collapse, which was not different between euploid and aneuploidy blastocysts (87% vs. 86%, $p = 0.1$). The median maximum volume increase of a single expansion event was 1.43 ± 1.09 nanoliters (nL), which is more than the volume of an average human zygote (0.9 nL, 120 μm in diameter). Interestingly, the median maximum volume increase in a single expansion event in euploid blastocysts was 34% higher than aneuploid blastocysts (1.61 vs. 1.07 nL, $p = 0.0002$). The average volume increase of a single expansion event was 0.56 ± 0.47 nL, with euploid blastocysts having a 23% higher average volume increase during a single expansion than aneuploid blastocysts (0.59 vs. 0.45 nL, $p = 0.0002$). During single expansion events, euploid blastocysts, compared to aneuploid blastocysts, also had a faster maximum rates (0.46 vs. 0.38 nL/hour, $p = 0.006$) and average expansion rates (0.28 vs. 0.24 nL/hour, $p = 0.02$). When collapses were included, the overall expansion rate was 0.15 ± 0.10 nL/hour, with euploid blastocysts having an overall faster rate than aneuploid blastocysts (0.16 vs. 0.12 nL/hour, $p = 0.002$).

Limitations, reasons for caution: This is an observational study performed in a single centre, and therefore the clinical significance of the finding needs to be validated in a multi-centered study with a larger sample size.

Wider implications of the findings: This is the first study that quantitatively and systematically characterized expansion dynamics for human blastocysts. The automatic extraction of the dynamic parameters provides an efficient tool for future studies, which may shed new light on the basic biology of human blastocysts.

Trial registration number: Clinicaltrials.gov, NCT01635049.

P-251 Predictive value of blastocyst morphology beyond aneuploidy testing: evidences from a randomized non-selection study

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Study question: Does blastocyst stage standard morphology assessment enhance embryo selection beyond aneuploidy testing?

Summary answer: Blastocyst morphology based selection does not significantly enhance live-birth rate in pre-implantation genetic diagnosis cycles for aneuploidy testing (PGD-A) nor predict adverse pregnancy outcomes.

What is known already: Trophectoderm (TE) biopsy and aneuploidy testing is increasingly used to enhance embryo selection in IVF cycles. However, beyond euploidy, no other parameters have been assessed to be used in combination with aneuploidy testing to further enhance the clinical management of PGD-A cycles. Blastocyst morphological evaluation has been extensively validated to predict implantation in IVF cycles and this association has been recently linked to its moderate predictive value on blastocyst aneuploidies. However, it is still unknown whether euploid blastocysts with different morphologies and development implant at a different rate or provide a different risk for biochemical losses, miscarriages or gestational complications.

Study design, size, duration: Randomized non-selection study preformed between December 2013 and December 2014 including all consecutive blastocyst stage PGD-A cycles performed in infertile patients of advanced female age (>35 years). In the randomization period, whenever more than one euploid blastocyst of different morphology was obtained, the embryo selection was subjected to randomization to rule out patient's specific factors (Study group). A subsequent follow-up period (Genuary 2015) where the selection between euploid embryos was based on blastocyst morphology was included (control group).

Participants/materials, setting, methods: ICSI and embryo culture in 5% O2/6% CO2. Prior to trophectoderm biopsy, morphology was assessed and categorized [excellent (≥3AA; group A)/good (3, 4, 5, 6, AB and BA; group B)/average (3, 4, 5, 6 BB, AC and CA; group C)/poor quality (≤3BB)] according to Gardner/Schoolcraft's criteria. Developmental rate was defined according to the day of biopsy post-fertilization (day 5, 6 or 7). Primary outcome was live-birth rate/transfer. Biochemical-losses, miscarriages and gestational outcomes were also compared between morphological classes using logistic regression models adjusted for potential confounding factors.

Main results and the role of chance: In the randomization period 171 cycles were excluded from the analysis since only one euploid blastocyst was obtained. 339 single euploid embryo transfers in 196 patients (female age 37.2 ± 3.1) were subjected to randomization since more than one euploid blastocyst (mean per cycle 2.8 ± 1.1) with different morphology ($N = 199$; 104 excellent, 40 good, 41 average, 20 poor) or with different time of development ($N = 159$; 73 day 5, 72 day 6, 14 day 7) was obtained for that cycle. Logistic regression analysis showed that poor morphology class ($p < 0.01$; OR = 0.09, 95%CI = 0.01–0.77; live-birth rate: 45.2%, 42.5%, 48.8% and 7.1%, for excellent, good average and poor, respectively) and day 7 embryos ($p < 0.01$; OR = 0.16; 95%CI = 0.03–0.85; live birth rate: 47.9%, 33.3% and 14.3% for day 5, 6 and 7, respectively) were significantly associated with reduced live-birth rate. Female age,

sperm parameters, previous IVF failures and miscarriages showed no association. Logistic models showed that morphology and day of development were not related to biochemical losses, miscarriages and gestational outcomes. In the follow-up period, 151 euploid SET were performed where morphological selection was possible. Even though higher percentage of excellent/good quality blastocysts were transferred in the selection group (76% vs 64.5%, respectively, $p < 0.01$), this did not translate in improved live-birth rate (46.3% vs 41.9% respectively; $p = 0.2$).

Limitations, reasons for caution: Not all morphological classes were represented in each cycle when the randomization was performed. Not all euploid embryos from a stimulation cycle were transferred. Even if based on a large dataset, this study is still not sufficiently powered to exclude moderate associations between blastocyst morphology and biochemical losses, miscarriages and neonatal outcomes.

Wider implications of the findings: This study provides class-one data about the limited value of blastocyst morphology selection beyond euploidy. Only very poor morphology and day-7 blastocysts showed lower potentiality. These embryos are usually discarded. However, when euploid they still provide live-births with no increase adverse outcomes strongly suggesting their use in PGD-A cycles.

Trial registration number: ISRCTN81216689.

P-252 Viral-positive patients: treatment outcomes and embryo morphokinetics

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Study question: Examination of IVF & ICSI treatment outcomes in patients with Hepatitis B, C and Human Immunodeficiency Virus (HIV) and its relationship with early embryo morphokinetics.

Summary answer: Pregnancy rates were significantly lower in viral-positive cohort, together with slower early embryo development for both viral-positive males and females.

What is known already: Time-lapse monitoring allows examination of embryo morphokinetics in various conditions that could impair fertility such as environment, lifestyle, drug effect or disease. Human immunodeficiency virus (HIV), hepatitis C virus (HCV) and hepatitis B (HBV) carriers are at risk of both reduced fertility and the risk of viral transmission to the partner or child. Published data is scarce regarding the fertility outcomes and embryo development characteristics in viral-positive couples, concentrate mostly on safety of the procedure. To our knowledge, morphokinetic analysis of embryos obtained from viral-positive patients has not been previously performed.

Study design, size, duration: Fifty viral-positive patients (24 HBV, 12 HCV, 14 HIV) who underwent fertility treatment over 12 months period were matched to controls. Data were analysed depending on the viral-positive status, origin of the disease (male, female) and treatment type (IVF, ICSI). 122 embryos from viral positive patients were compared against 139 control embryos examining only embryos that were transferred or cryopreserved.

Participants/materials, setting, methods: Controls were matched according to female age at oocyte retrieval (±12 months), treatment type, stimulation protocol and number of oocytes collected (±2). One control was matched to each viral-positive case. Viral-positive group was cultured in a standard incubator with PrimoVision® system, whereas control group was cultured in EmbryoScope® incubator. Morphokinetic data collected for early embryo development was: tPNf (pronuclear fading), t2, t3, t4, t5 (time to cell division).

Main results and the role of chance: Clinical pregnancy rates were reduced for viral-positive couples vs control (26% and 48%, $p = 0.012$, McNemar test for matched pairs), with an odds ratio of 0.380 (95% CI 0.16–0.88) ($p = 0.023$, Pearson's Chi-square). Significantly reduced clinical pregnancy rates were found when male but not female was viral-bearing when compared to control (25% vs 53.1% in males, $p = 0.021$; 25% vs 35% in females, $p = 0.490$, Pearson's Chi-square). Survival analysis (Kaplan-Meyer method and log-rank tests) were performed to analyse morphokinetic parameters. A significant difference in the kinetics of early divisions was observed, with embryos from viral-positive patients reaching the 2-cell ($p = 0.0001$), 3-cell ($p = 0.025$) and 4-cell stages ($p = 0.030$) slower than embryos from control patients. When early division kinetics were analysed separately for each virus-bearing gender, in both males and females embryos divided significantly slower compared to the controls, with the differences being more pronounced in males up to t5 ($p < 0.05$ for t2, 3, 4, 5, log-rank test), whereas in females the differences were decreasing ($p < 0.05$ for t2, t3, t4, log-rank test) as divisions progressed, and were lost by the t5 ($p = 0.727$, log-rank test).

Limitations, reasons for caution: The study is limited by a low number of participants, which at this stage precluded a thorough analysis of each viral condition separately. Morphokinetic parameters were determined using two different time-lapse imaging systems, which could have affected the data collected, although the imaging time-frames were the same.

Wider implications of the findings: These data suggest that the presence of a virus during an assisted conception cycle may compromise embryo development and that this compromised development may be implicated in reduced pregnancy outcome. Furthermore, it may be prudent to consider development of morphokinetic embryo selection algorithms specifically for patients with known viral-positive infections.

Trial registration number: N/A.

P-253 Development of a non-invasive method for selection of human embryos with high implantation potential based on Fourier transform Infrared (FT-IR) spectroscopy and artificial neural networks

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Study question: Does FT-IR spectroscopy of spent culture embryo supernatants provide an OMICS–like view of the chemical/biochemical status of the embryo metabolism to be used to evaluate embryo implantation potential?

Summary answer: Multivariate analysis of metabolomic spectral patterns of human IVF embryos developed by an artificial neuronal network system could identify an embryo with high implantation potential.

What is known already: The metaboloma is the chemical print produced in a biological system, representing its functional phenotype, and consists of a set of 2500 to 3000 molecules and potential biomarkers. Metabolomic techniques were used as non-invasive approaches for IVF studies analyzing different metabolites in embryo-culture supernatants. Particularly, near infrared spectroscopy (NIR) has been used to evaluate embryo pregnancy ability. Although these results were initially encouraging, a recent randomized clinical trial did not show benefits of NIR over conventional morphological evaluation. FT-IR has been widely used to study the biochemical composition of biological materials. Nevertheless it has never been applied in IVF technology.

Study design, size, duration: Prospective cohort study, enrolling unselected patients undergoing IVF or ICSI cycles from November 2012 to December 2014. Four hundred fourteen patients were enrolled, 400 of them reached egg retrieval, 346 had appropriate ova for fertilization an 294 achieved embryo transfer (268 on day 3, 26 on day 5). This is an ongoing collaborative project between a private practice ART clinic and a University Research Center.

Participants/materials, setting, methods: Participants with up to 3 previous IVF cycles, and up to 41 years of age were excluded. Embryos were cultured individually in trigas incubators, and 1 or 2 embryos were transferred under conventional clinical and embryological criteria on day 3 or 5. An aliquot of spent culture media was frozen in liquid nitrogen for future spectral acquirement and data analysis. A total of 497 embryos were transferred on day 3, and 38 on day 5.

Main results and the role of chance: Five hundred and thirty-five embryos were transferred, and 136 of them implanted (25.4%). After thawing, the sample was centrifuged for oil extraction, droplets were transferred to a ZnSe plate, and vacuum dried for 40 min. Due to technical problems with oil extraction in day 5 embryos, only day 3 embryos were finally evaluated. Spectra coming from a total of 1315 embryos, comprising the 497 day 3 transferred embryos and the non-transferred, vitrified embryos, were used to construct the database. Spectra from transferred embryos were classified as Class I (belonging to full implantation embryos, i.e., singletons in sET or twins in DET), Class II (non implanted) and Class III (50%, 1 of 2 embryos implanted). Multivariate methods were applied in order to obtain a model able to discriminate spectra belonging to Class I from Class II supernatants, using ***Opus 3.1*** and ***4.5*** software (Bruker Optics, Germany), that include the IDENT package for cluster analysis and principal component analysis and ***NeuroDeveloper***® (Synthon Analytics, Germany) for the development of artificial neural networks applied in the construction of

libraries and other databases. This showed a 76% accuracy rate, 75% sensitivity and 76% specificity in predicting implantation.
Limitations, reasons for caution: Due to the characteristics of ANNs, needing a huge number of cases to "learn" during the process, clinical validation of these results still need a larger dataset of cases, to increase accuracy through further analysis of full implantation cases (singletons in sET, or twins after DET)
Wider implications of the findings: Addition of other non-invasive embryo selection techniques like morphokinetics could improve the accuracy rate and positive and negative predictive value of metabolomic assessment and help in the construction of predictive models to improve embryo selection and secondarily pregnancy rate.
Trial registration number: Non randomized trial.

P-254 Effect of dual trigger with gonadotropin-releasing hormone agonist (GnRH-a) and human chorionic gonadotropin (hCG) in embryo quality

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Study question: Does dual trigger of final oocyte maturation improve the embryo quality in IVF cycles?
Summary answer: Co-administration of GnRH-a and hCG for final oocyte maturation does not significantly modify embryo quality in IVF cycles.
What is known already: Oocyte maturation for IVF cycles is commonly induced by hCG as a surrogate for the natural LH surge. In the last years, the use of GnRH-a for final follicular maturation has been shown to significantly reduce the occurrence of ovarian hyperstimulation syndrome compared with hCG triggering, however, a poor reproductive outcome was reported after GnRHa triggering. More recently, the so-called "dual trigger" that combines GnRH-a with hCG has been investigated in IVF with promising results.
Study design, size, duration: Prospective randomized study. The population under study consisted of 73 consecutive patients treated by IVF or ICSI at Hospital Donostia Assisted Reproduction Unit, from January to March 2014. Participants were randomly assigned to two groups: 1) hCG trigger group ($n = 39$) and 2) dual trigger (GnRH-a + hCG) group ($n = 34$).
Participants/materials, setting, methods: Final oocyte maturation was triggered by either 250 μg of recombinant hCG (Ovitrelle; Merck Serono) alone, or by 250 μg of recombinant hCG plus 0.2 mg of triptorelin (Decapeptyl; Ipsen Pharma), depending on the assigned group. The embryo morphology classification criteria of the Association for Reproduction Biology Studies (ASEBIR, according to its initials in Spanish) were followed. All embryo transfers were performed 48 h after oocyte retrieval. Statistical analysis was performed using SPSS 21.0.
Main results and the role of chance: Female mean age, corporal mass index, basal FSH levels, number of antral follicles and AMH levels, as well as cycle stimulation characteristics (estradiol, progesterone) were similar in both groups. We found a higher proportion of good quality embryos transferred in patients who received the double trigger compared to hCG trigger group (43.8% vs 35% respectively). However, the differences were not statistically significant.
Limitations, reasons for caution: Our results are limited by the small sample size; therefore, it was not possible to analyze the effects of dual triggering in IVF results of different subgroups of patients. A larger data set is needed to provide more information about dual triggering potential use. This is not a blinded study.
Wider implications of the findings: The results from the present study indicate that the transferred embryo quality was not modified by dual trigger of final oocyte maturation. However, further large prospective studies are needed to elucidate the aforementioned recommendation.
Trial registration number: Not applicable.

P-255 Embryos with delayed development at day 5 must be frozen. Improving implantation rates of early blastocysts after elective vitrification

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Study question: What are the best endometrial conditions for the transfer of embryos delayed in development during the culture to the blastocyst stage? (synchronous vs substitutive cycle).
Summary answer: Embryos with good morphology but delayed development benefit for elective FET with customized endometrial preparation increasing their chances to implant.
What is known already: There are growing evidence about the impairment on the endometrial receptivity caused by ovarian stimulation. The advanced histology and down regulation of the progesterone receptor are suspected indicators of an advanced receptive phase with effect on embryo-endometrium asynchrony causing implantation failure. In view of these facts, "freezing all" strategies have been proposed but is controversial because its use makes treatments longer in time and more expensive increasing the burden supported by the patients.
Study design, size, duration: Retrospective analysis of transfers performed in our institution from January 2013 to September 2015 in patients using their own oocytes. For the analysis, we select only cases that receiving 1 or 2 embryos with the same stage of development at day 5: 159 ET with embryos classified as early (grade 1 Istanbul Classification) and 601 transfers where only good quality blastocyst were transferred (grade 2, 3 or 4 Istanbul Classification).
Participants/materials, setting, methods: All the cases included were patients using their own oocytes and transferred at day 5 of embryo development. We made two groups, embryos delayed (grade 1), comparing fresh and frozen-thawed transfers, and good quality embryos (grade ≥ 2), fresh and frozen-thawed transfers. We compared implantation rates between transfers in fresh and after freezing. Chemical and clinical pregnancies and miscarriages rates were analysed as secondary endpoints. Logistic regression and Pearson's χ^2-test were applied to evaluate differences.
Main results and the role of chance: We analysed a total 159 transfers of delayed embryos, 105 fresh and 54 freeze-thawed transfers. We observe an increase in implantation rate (9.77% vs 30.34%, $p = 0.00002$), clinical pregnancy (12.5% vs 44%, $p = 0.0003$), positive test (21.0% vs 46.3%, $p = 0.005$) and miscarriages (15.4% vs 40.0%, $p = 0.085$) in freeze-thawed transfers. We try to verify if the same effect occurs in good quality embryos transfers. A total of 601 treatments were analysed, 488 fresh and 113 freeze-thawed transfers. We can't observe differences between implantation rate (28.57% vs 27.07%, $p = 0.69$), clinical pregnancy (37.4% vs 39.8%, $p = 0.65$), positive test (50.0% vs 54.9%, $p = 0.89$) and miscarriages (14.0% vs 27.6%, $p = 0.026$).
Limitations, reasons for caution: The main limitation is that this is a retrospective analysis.
Wider implications of the findings: Embryos with slower development in the culture may be falsely classified as low-implantation capacity when the problem really is the endometrial asynchrony. These embryos have higher implantation rates in a FET with substitutive endometrial preparation and must be electively frozen.
Trial registration number: No trial registration number.

P-256 Comparison of implantation rate and pregnancy rates of frozen/thawed embryos at blastocyst stage and frozen/thawed embryos at cleavage stage, cultured for transfer on blastocyst stage

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Study question: Implantation rate (IR) and clinical pregnancy rates (CPR) of frozen/thawed embryos at blastocyst stage versus thawed embryos at cleavage stage, cultured for transfer on blastocyst.
Summary answer: Neither the IR nor the CPR differed between both groups. However more embryos were needed to obtain pregnancies when embryos were frozen at cleavage stages.
What is known already: Cochrane data base information published in 2013 established that fresh blastocyst stage transfers improved IR rates, but it is unknown if this difference persists with frozen/thawed embryos transferred on blastocyst stage.
Study design, size, duration: A retrospective study of 147 thawing cycles was performed from January 2009 to December 2015.

Participants/materials, setting, methods: We compared the outcomes of 49 thawing cycles done with embryos at blastocyst stage (Group A) with 98 thawing cycles where embryos at cleavage stage (day 2 or day 3) were thawed and grown to blastocyst stage (Group B). All frozen embryos were surplus embryos from IVF cycles with one fresh embryo transfer.
Main results and the role of chance: A total of 493 embryos were thawed, 90 at blastocyst stage and 403 at cleavage stage. In Group A, 74% of the embryos were replaced (67/90) versus 40% in Group B (161/403) ($p < 0.001$). The mean number of embryos thawed for transfer was four times larger in Group B (4.11) than Group A (1.84). Furthermore, the mean number of blastocyst transferred was also larger in Group B (1.64) than Group A (1.36) ($p > 0.001$). However, no significant differences were found neither for IR (18% in group A versus 16% in Group B) ($p > 0.05$) nor CPR (18% in group A and 11% in group B) ($p > 0.005$). Twenty five clinical pregnancies were obtained, 9 in Group A (6 singleton, 3 multiple) versus 16 in Group B (14 singleton, 2 multiple).
Limitations, reasons for caution: The main limitation was the low number of thawing cycles in Group A, so results need to be confirmed in larger studies. Embryos replaced were surplus embryos of IVF cycles with one fresh embryo transfer, so their quality could be suboptimal.
Wider implications of the findings: Due to no significant differences in CPR, transfer of frozen/thawed embryos in blastocyst stage could reduce the surplus of cryopreserved embryos, selecting for freezing only those embryos with more chances to obtain pregnancies.
Trial registration number: Not applicable.

P-257 Is there a room for lowering miscarriage rates in time-lapse cycles?

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Study question: What is the probable reason for consistent miscarriage rates after applying time-lapse techniques?
Summary answer: Overall miscarriage rates while using time-lapse techniques could be achieved by inappropriate embryo selection.
What is known already: Time-lapse observation of embryo development from zygote stage to blastocyst stage is believed to be an effective tool in embryo deselection and, thus, could improve pregnancy rates. But the recent data remains controversial and needs to be verified. Still, miscarriage rates after performing time-lapse techniques is comparable with so called "conventional" incubation.
Study design, size, duration: For this retrospective cohort study we have analysed pregnancy outcomes after 579 embryo transfers (cycles) of 572 patients in the three-year period. Oocyte donation cycles were excluded from the study. All embryos were cultivated within commercial time-lapse incubator. Fabricator's recommendations were used for further embryo selection. Only first attempt (embryo transfer) of each cycle was included to this study. ICSI or IMSI were applied in all cases.
Participants/materials, setting, methods: Patients were aged 23 to 44 years (mean age 34.0 ± 4.5 years). We have compared three groups of patients: Group A – cycles, where array comparative genomic hybridization (aCGH) was performed with the consideration of morphokinetic features of embryo development. Group B – Fresh Day 5 selected embryo transfer (ET). Group C – Cryo Day 5–7 selected ET. One or two good quality blastocysts were transferred on day 5, 6 or 7 regarding to Group.
Main results and the role of chance: Although the ongoing pregnancy rates were similar within all three groups (Group A – 48.6% vs Group B – 38.9% vs Group C – 46.9%, NS), miscarriage rate in aCGH group was dramatically lower (Group A – 2.7% vs Group B – 11%, $p < 0.05$ and Group A – 2.7% vs Group C – 12%, $p < 0.05$), whereas there was no statistically significant difference between Groups B and C. Euploid embryos, mostly, show strict binary behaviour: they implant or do not implant, whereas aneuploid embryos maintain the ability to implant causing early pregnancy loss (7–12 weeks). Thereby, the possible reason for relatively high miscarriage level while using time-lapse techniques is the accidental selection of aneuploid embryo. For now, time-lapse techniques cannot overcome their limitations in choosing the euploid embryos and substitute the performing of preimplantation genetic screening.
Limitations, reasons for caution: In this study, we did not include cumulative pregnancy rates and biochemical pregnancy was stated as the absence of pregnancy. It should be taken to consideration that aCGH group is consisted of poor-prognosis patients with no regards to their age.
Wider implications of the findings: Long-term cultivation itself doesn't have an adverse effect on embryo implantation and further development. There is obvious need in creating better algorithms for embryo selection by time-lapse techniques.
Trial registration number: None.

P-258 Using Eeva combined with morphology to select a single blastocyst for transfer achieved higher live-birth rates compared to using morphology alone: a matched case-control study

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Study question: To what extent can live birth rates be improved by using the Eeva Test to help select a single blastocyst for transfer?
Summary answer: Patients using the Eeva Test with morphology to select a single blastocyst for transfer achieved higher live birth rates compared to patients using morphology alone.
What is known already: Improved embryo assessment will help increase the utilisation of elective single embryo transfer (eSET). Preimplantation genetic screening is effective in selecting euploid embryos, showing promise in improving live birth rate, however, it is invasive and not available in certain countries. Time-lapse technology has been recently applied to clinical embryology practice, but definitive clinical data to demonstrate improved live birth outcome is still needed. Here we attempted to assess to what extent using the Eeva Test, an automated time-lapse technology that measures early cleavage timings, could improve pregnancy and live birth rates for Day 5 eSET.
Study design, size, duration: A retrospective matched case-control study was conducted in a single IVF centre between September 2012-September 2014 Four hundred and thirty one patients undergoing their first IVF cycle with Day 5 eSET were included in the study.
Participants/materials, setting, methods: A total of 114 patients used the Eeva Test. Both Eeva results and morphology were used to select a single blastocyst for transfer. Three hundred and seventeen control patients, where only morphology was used for embryo selection, were propensity-matched to the Eeva group based on age, number of eggs retrieved, and number of fertilised eggs (2PN). Clinical pregnancy was defined by fetal heartbeat at 6–8 weeks by ultrasound. Live birth was confirmed for each patient.
Main results and the role of chance: Following propensity matching, patient age, number of eggs retrieved and number of 2PN per patient were similar between the Eeva Test vs matched-control Group (age: 33.2 ± 3.8 vs 33.0 ± 3.8, $p = 0.6$; number of eggs: 12.3 ± 5.3 vs 13.0 ± 5.0, $p = 0.3$; number of 2PN: 7.7 ± 4.0 vs 7.3 ± 3.8, $p = 0.4$). However, patients using the Eeva Test achieved significantly higher rates of clinical pregnancy (49%, 56/114, vs 38%, 119/317, $p = 0.035$). Furthermore, follow-up of patients' live birth outcome revealed that patients in the Eeva Group had a 45% relative increase in live birth rate compared to that of the matched-control group using morphology alone (45%, 51/114, vs 31%, 99/317, $p = 0.012$).
Limitations, reasons for caution: This study is not a randomised controlled trial (RCT). In addition, the control patients had embryos cultured in conventional incubator without time-lapse monitoring.
Wider implications of the findings: This study is the first to show that using the Eeva Test may improve live birth rates for Day 5 eSET, validating the relationship of early cleavage timings to embryo viability. As the Eeva Test is non-invasive and easy to adopt, it may facilitate the practice of eSET more broadly.
Trial registration number: None.

P-259 Impact of male BMI on IVF outcome after elective single blastocyst transfer. A prospective study

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Study question: Does male Body mass index (BMI) affect IVF outcome after elective single blastocyst transfer (eSBT)?

Summary answer: Raised male BMI does not negatively impact the clinical pregnancy rate in IVF cycles in which a good quality single blastocyst is electively transferred.
What is known already: Whilst raised female BMI (≥25 kg/m^2) has been associated with a negative effect on IVF outcome, there is conflicting evidence regarding the impact of male BMI. Most of the available studies were retrospective, used self-reported male BMI records and included outcomes following transfer of embryos at various stages of development without adjusting for important confounders.
Study design, size, duration: This is a prospective observational study conducted in a tertiary assisted conception unit in teaching hospital between June 2015 and January 2016. Both male and female BMI measurements were recorded at the beginning of the treatment cycle. Cycles with self reported BMI, cycles in which frozen or surgically-retrieved sperm, donor gamete or PGD was used, embryo transfer took place prior to the blastocyst stage or more than one blastocyst transferred were excluded from the analysis.
Participants/materials, setting, methods: Couples with female age below 40 years, having one cycle of IVF/ICSI with eSBT during the study period were included. All blastocysts transferred were of good quality (grade 3CC or higher). Patients were divided into two groups according to male BMI; group 1 in whom male BMI was normal (18.5–24.9 kg/m^2), group 2 in whom male BMI was raised (≥25 kg/m^2). BMI was objectively measured for all couples by trained nurses on two equally calibrated machines.
Main results and the role of chance: A total of 163 couples were included in the study; 65 in group 1 (normal BMI) and 98 in group 2 (raised BMI). The pregnancy (51% vs 52%, p = 0.88), and clinical pregnancy (39% vs 41%, p = 0.87) rates were similar in the two groups, respectively. After adjusting for important confounding variables, including female age, female BMI, cause of infertility, baseline female serum AMH level, total gonadotrophin dose used during ovarian stimulation, number of oocytes retrieved and fertilised normally, method of fertilisation used (whether IVF or ICSI) and the number of surplus blastocysts cryopreserved following eSBT, the likelihood of achieving a clinical pregnancy in group 2 was not significantly different from that in group 1 (adjusted OR = 1.87, 95% CI 0.84 – 4.19, p = 0.13).
Limitations, reasons for caution: Although this was a prospective study with strict inclusion criteria and the results were adjusted for important confounders, it is a single-center study and the analysis was limited to cycles in which female age was below 40 years and elective single blastocyst transfer took place on day 5.
Wider implications of the findings: Our study suggests that raised male BMI does not impact IVF outcome after a good quality blastocyst transfer. Further research is needed to confirm these findings and impact of male BMI on live birth rate.
Trial registration number: Not Applicable.

P-260 Alginate-matrigel scaffold usage for in vitro development of preantral follicles

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Study question: In vitro culture (IVC) of ovarian follicles is a new strategy in reproductive technology which helps to a better understanding of the complicated folliculogenesis process.
Summary answer: Three dimensionally follicle culture through alginate-matrigel scaffold could mimic the ovarian tissue stroma regarding extracellular matrix components and similar morphologic growth.
What is known already: It has been achieved that proper selection of the matrix and also culture media could increase the survival and maturation rate of the follicles. Also, vitrification is an important step in preserving the ovarian tissue for later usage.
Study design, size, duration: To conduct the study, 250 ovaries from 12-day-old NMRI mice were distributed into control and vitrification groups.
Participants/materials, setting, methods: Ovaries of the vitrification group were vitrified by Needle Immersed method. At first, in both groups, ovarian tissue morphology was evaluated. In the second stage, preantral follicles (Mean diameter:120–140 μm) were mechanically isolated from control and vitrified ovaries, then encapsulated in alginate-matrigel scaffold and cultured for 12 days. Follicles survival, growth and maturation rate, also quantitative expression of oocyte maturation genes (*Gdf9*, *Bmp15* and *Fgf8*) and protein expression were assessed.
Main results and the role of chance: Morphological integrity of vitrified warmed ovaries was well preserved as like as the fresh control one. Although survival rate of culture preantral follcles in control group was significantly higher than the vitrification one (94% versus 81.6%), antrum formation was similar in both groups. Also, during culture period, follicle diameter was significantly increase in both groups. In assessment of oocyte maturation genes expression during 12 days of follicle culture, the decreasing pattern was observed in both groups. No significant difference was observed between control and vitrification groups at the first and last days of culture in genes expression. This trend was assured by protein expression evaluation (*Gdf9* and *Bmp15*) which were indicated sharp staining in 12 days of culture in both groups. At the end, maturation rates were indicated a non significant difference between control and vitrification groups (32% versus 33%).
Limitations, reasons for caution: None.
Wider implications of the findings: Considering the same outcome of vitrification group compared to control one, as well as the supporting role of alginate-matrigel matrix in preantral follcle development and subsequently maturation, it seems that this scaffold could be an acceptable one for preantral follicle in vitro culture.
Trial registration number: Registration number is not required for this study.

P-261 Study of Tnp1, Tekt1 and Plzf genes expression during in vitro three dimensional neonate mouse testis culture

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Study question: Can post-meiotic genes express in the three dimensional testis culture?
Summary answer: *Tnp1* as a post-meiotic gene can be expressed during in vitro three dimensional neonate mouse testis culture.
What is known already: In vitro spermatogenesis has a long research history beginning in the early twentieth century. This process did not proceed beyond the meiotic pachytene stage. The organ culture method was therefore abandoned, and alternative of cell culture methods were then chosen by many researchers. Study of the genes involved in different stage of spermatogenesis are very important. Here we assessed whether *Tnp1*, *Tekt1* and *Plzf*, with crucial role in spermatogenesis can express during organ culture of testis. So, we used organ culture of neonate mouse to understand of spermatogenesis process in molecular level during 12 weeks culture.
Study design, size, duration: Sampling procedures: 10 mouse pup, testis were removed. The tunica was removed after fully exposing the seminiferous tubules. The testis tissue was separated into smaller pieces of seminiferous tubules. The size of the pieces was arbitrary, approximately 1 mg in weight or 1 mm^3 in size when compacted. One to three testis tissue fragments were transferred to the hexahedrons, and incubated by placing the 6-well plates in a culture incubator. And cultured for 12 weeks.
Participants/materials, setting, methods: Total RNA was extracted from the 12 weeks 3D cultured tissue of neonatal mouse. The cDNAs were synthesized. For PCR reactions, primers were adapted from other primers (designed by the NCBI website). The quality of the PCR reactions was confirmed by melting curve analyses. Efficiency was determined for each gene using a standard curve. For each sample, the target genes were normalized to a reference gene and calibrated to an adult or neonatal testis.
Main results and the role of chance: The results showed that expression of *Tekt1* as a mitotic gene decreased comparing to adult mouse testis, significantly ($p \leq 0.05$). Meanwhile expression of *Tnp1*, as meiotic gene, increased significantly comparing with neonate mouse testis in beginning of culture ($p \leq 0.05$). Expression of *Plzf* showed no significant difference during 12 weeks culture ($p \geq 0.05$). Histological study showed only different types of spermatocytes and post-meiotic stages of germ cells could not be detected. This kind of three dimensional culture can induce expression of post-meiotic gene, *Tnp1*, but May it remains in molecular level and could not pass beyond meiosis.
Limitations, reasons for caution: The limitation of research is that only gene expression is not strong tool for assessing the functionality and it is necessary to have final product, functional spermatozoa, to interrupt about cultural system.

Wider implications of the findings: Previous studies in the in vitro spermatogenesis using an organ culture technique are few. Recently, Yokonishi et al. (2013) used 6 – 8 weeks of culture and evaluated spermatogenesis by histological and Immunohistochemical techniques.
Trial registration number: it is basic science.

P-262 The impact of laser assisted hatching in an oocyte donation program using egg cryobanking: a prospective, randomized, comparatrive study

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Study question: Is beneficial the performance of laser assisted hatching prior transfer in embryos derived from aseptically vitrified/warmed oocytes in an oocyte donation program?
Summary answer: Clinical results is significantly improved when laser assisted hatching is being performed in embryos which derived from vitrified/warmed oocytes.
What is known already: Lately, studies show very promising results after vitrification of oocytes. However, a recent review on oocyte vitrification (Potdar et al., 2014) shows great heterogeneity in the reported clinical results after oocyte vitrification and thus highlights that there is space for improving the clinical outcome. An important parameter that could influence negatively the clinical outcome of the vitrified oocytes is the hardening of their zona after vitrification/warming procedure and the failure of the resulting embryos to hatch and implant. There are not enough studies which evaluate the clinical impact of assisted hatching in human embryos deriving from vitrified oocytes.
Study design, size, duration: A prospective, randomized, comparatrive study was performed from January 2015 to December 2015. All cases were participating in oocyte donation program of Iakentro IVF Center. Two groups were under study. In group A we included cases were embryos derived from donated, vitrified oocytes were transferred without assisted hatching. In group B we include cases in which embryos derived from vitrified oocytes were hatched prior transfer using a laser system.
Participants/materials, setting, methods: The two under study groups were formed after a randomization procedure. Seventy cases were included in each group. In all cases, recipients were donated oocytes which were vitrified using a closed system vitrification. After warming, oocytes were fertilized and cultured to day 5. All transfers were performed on day 5 (blastocyst stage). Group A embryos were transferred without assisted hatching. Group B embryos were hatched with the use of laser pulses 2 h before transfer.
Main results and the role of chance: Pregnancy rate (48.5% vs 74.28%), clinical pregnancy rate (45.7% vs 68.5%) and ongoing pregnancy rate (40.2% vs 66.5%) were significantly higher in group B. Furthermore implantation rate was significantly higher in group B (29.2% vs 58.8%). In fact the IR is doubled when laser assisted hatching was used. This study shows that laser assisted hatching is beneficial for embryos that derive from aseptically vitrified oocytes.
Limitations, reasons for caution: This study includes cases in which donated oocytes from healthy fertile women were used. The results of this study should not be extrapolated to any other female group.
Wider implications of the findings: The findings of this study are very interesting: vitrification/warming procedure of oocytes could induce hardening of the zona pellucida of the resulting embryo and this could affect the clinical outcome. The use of a laser assisted hatching before embryo transfer should be highly recommended in oocyte cryo cycles.
Trial registration number: The study was approved by the Institutional Review Board (Ref. no. 3/2015, granted 7 January 2015).

P-263 Are embryo morphokinetics affected by patient age?

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Study question: When twelve morphokinetic parameters are examined from utilised embryos, can differences in two patient age groups be seen and could these differences be used to improve current embryo selection methods?
Summary answer: Variations in morphokinetic parameters can be seen between two age groups; younger patient's embryos have a significantly different morphokinetic profile to those from older patients.
What is known already: With the advent of time-lapse systems, many are seeking to determine more effective mechanisms for embryo selection. Data presented thus far suggests that treatment, environment and patient parameters can affect an embryo's early morphokinetic profile. However, these apparent differences are yet to be used to aid embryo selection clinically. In addition, to our knowledge, there is yet to be a large cohort of embryos analysed for apparent morphokinetic differences when considering patient parameters such as age.
Study design, size, duration: Images from embryos cultured in an EmbryoScope® incubator over a 40-month period were retrospectively analysed. Analysis of two patient groups (younger; <35, older; ≥35) comprised two stages; differences in morphokinetic parameters in all utilised embryos (transferred or cryopreserved); differences in morphokinetic parameters in embryos that created a fetal heartbeat.
Participants/materials, setting, methods: Embryos (n = 2069) from patients undergoing treatment at a single study site were included. Culture comprised Vitrolife sequential media (G1-Plus™ and G2-Plus™) at 5% O_2, 6% CO_2, 37°C throughout in an EmbryoScope® incubator. Morphokinetic data for twelve parameters (tPNf (pronuclei fading), time to n cell (tn) (t2, t3, t4, t5, t6, t7, t8, t9+), time to morula (tM), start of blastulation (tSB) and blastocyst (tB) were derived retrospectively and collated for analysis.
Main results and the role of chance: The morphokinetic profiles (shown as median hours post-insemination for tPNf and median hours post-tPNf for all other morphokinetic parameters) of utilised embryos from younger patients were statistically significantly different to those from older patients when considering tPNf (23.3 vs 24.0), t3 (13.6 vs 13.8), t4 (14.3 vs 14.5), t5 (26.6 vs 27.0), t6 (27.7 vs 28.1), t7 (29.0 vs 29.3), t9+ (46.8 vs 47.5), tM (58.5 vs 59.9), tSB (71.1 vs 72.5) and tB (81.8 vs 83.4) (significant at $p < 0.05$, Mann-Whitney U test). In addition, embryos that created a fetal heartbeat displayed statistically significant morphokinetic differences between younger and older patients when considering tPNf (23.4 vs 23.8), t3 (13.6 vs 13.8), t5 (26.3 vs 26.7) and t6 (27.2 vs 27.8) (significant at $p < 0.05$, Mann–Whitney U test). Overall, embryos from younger patients appear to develop faster than those from older patients.
Limitations, reasons for caution: Morphokinetic analyses are, by their nature, subjective therefore caution should be taken. This study is a retrospective analysis and prospective trials are required to elucidate the effect of including patient age in embryo selection algorithms. Further analyses will be performed to investigate the significance of cell cycle length and synchrony.
Wider implications of the findings: These data suggest that an embryo's morphokinetic profile can be affected by patient age. These results could inform the development of patient age-specific embryo selection methods resulting in individualised treatment and ultimately an increased chance of selecting embryos with the highest implantation potential.
Trial registration number: n/a.

P-264 High implantation and pregnancy rate after blastocyst transfer in natural cycles

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Study question: The transfer of frozen blastocyst in natural cycles may increase the pregnancy and implantation rate?
Summary answer: High pregnancy and implantation rates are observed following transfer of frozen blastocysts, utilizing natural cycles.
What is known already: The high progesterone level before HCG in IVF cycles causes advanced endometrium. Therefore it is suggested that embryo cryopreservation for later transfer.
Study design, size, duration: Retrospective study to evaluate clinical pregnancy and implantation rates in women that transferred frozen blastocysts using natural cycle during the year of 2015 at Clinica Fertilitat.

Participants/materials, setting, methods: Forty-four women with no pregnancy when transferred fresh blastocysts, were submitted to transfer of one or two blastocysts using natural cycle. Five days after spontaneous ovulation, the embryo transfer were performed. No medication was utilized during this process.
Main results and the role of chance: The women average age was 33.6 years. The endometrium thickness was de 10.5 mm at the time of transfer. The pregnancy and implantation rate was the 72.7 and 59.1%, respectively. Twenty seven women transferred two embryos and 17 (38.7%) transferred only one embryo. Women that transferred only one embryo had a pregnancy rate of 52.9, while women that transferred two embryos, 85.2% were pregnant. The percentage of twins, abortion, and ectopic pregnancy were 37%, 18.7% and 12.5% respectively.
Limitations, reasons for caution: The natural cycle utilization, without the use of hormones, it is a brake of paradigmas with the patients that are used to mainly interventions in IVF procedures.
Wider implications of the findings: It seems that there is a negative effect of the hyperstimulation on the endometrium maturation, moving the implantation window. The natural cycle physiology seems to favor to a better synchrony to embryo implantation.
Trial registration number: Not necessary.

P-265 A prospective multicentre comparison of two different single step culture media using sibling embryos

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Study question: Can G-TL™, a CE-marked medium specifically designed for extended and undisturbed embryo culture, replace the clinics standard global® medium, supplemented with dextran serum substitute (DSS)?
Summary answer: As no statistical difference in blastocyst development was detected and a significantly lower miscarriage rate was observed with G-TL™ medium, there is evidence to support change.
What is known already: Single step embryo culture media do not generally require media changes during embryo culture, a feature which makes them attractive for time-lapse culture and minimises handling stress. There are several media for undisturbed culture commercially available; however, despite being well established and effective in IVF laboratories, all are not appropriately CE-marked. G-TL™(Vitrolife) is a complete medium that is CE-marked, as a class II medical device, providing regulatory compliance for the European market. Global® medium (LifeGlobal), supplemented with DSS (Irvine), is a non-CE marked combination. Increasing demands from regulatory bodies for approved products justifies this comparison of two media alternatives.
Study design, size, duration: The study was designed as a prospective multicentre sibling embryo study. Four clinics following standard laboratory protocol participated. The primary endpoint was blastocyst formation. Secondary endpoints included blastocyst utilisation and clinical outcomes. To improve the outcome of the primary endpoint by 12%, the number of blastocysts required was 482 (241 in each arm). The embryos recruited for the study were cultured between June 2014 and December 2015.
Participants/materials, setting, methods: 1033 embryos fertilized by both IVF and ICSI were included in the study. Embryos subjected to PGD or PGS were excluded. Allocation to the different culture media was done randomly at low magnification after fertilization check on day 1. Embryos were cultured in both standard incubators (Miri, ESCO) and in EmbryoScope® time lapse systems (Vitrolife). G-TL™ is a ready-to-use culture medium; global® medium was supplemented with DSS as a protein source.
Main results and the role of chance: 576 blastocysts developed from the 1033 2PNs. The data analysis revealed no significant difference in 2PN to blastocyst development on day 5 between G-TL™ and global®, 60.3% and 63.3% respectively ($p = 0.3455$). The results were also comparable between G-TL™ and global® when assessing good quality blastocyst formation (GQB) per 2PN (equivalent to 3BB or better using the Gardner score) (19.9% and 20.2%, $p = 0.9447$). Analysing GQB per blastocysts formed, results were 31.7% and 28.0% ($p = 0.5406$) for G-TL™ and global® respectively. Utilisation rate of blastocysts (transferred or cryopreserved) per 2PN for G-TL™ and global® were 43.3% and 45.7% respectively ($p = 0.4870$).)

Interestingly, there was, a significantly lower miscarriage rate for G-TL™, 5.0% compared to 17.7% for global®/DSS ($p = 0.0279$) for all embryo transfers ($n = 122$). The clinical pregnancy rate per transfer (53.3% and 54.8%, $p = 0.8681$) and implantation rate (48.5% and 45.6%, $p = 0.7209$), were comparable between G-TL™ and global®.
Limitations, reasons for caution: The study did not reach the targeted difference for the primary endpoint. Assessing blastocyst formation as primary outcome may not relate to clinical outcome, however, since miscarriage rate reached statistical significance, aside from meeting regulatory compliance, introduction of G-TL® may minimise the burden of miscarriage for patients in participating clinics.
Wider implications of the findings: No significant difference in blastocyst formation between G-TL™ and the global®/DSS combination was seen but the results support the use of G-TL™ as a single step culture medium for markets where regulatory compliance is required, compared with global®/DSS for embryo culture and avoids the need for protein supplementation.
Trial registration number: not applicable.

P-266 Correlation of cumulus gene expression of AKT1, BCL2L2 and SHC1 with murine oocyte maturation

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Study question: Could be cumulus cells (CCs) gene expression profile explored as potential markers of oocyte maturation?
Summary answer: Data have shown a CC gene expression associated with oocyte maturation timing.
What is known already: It is widely recognized that the production of competent oocytes is related to the bidirectional communication existing between the oocyte and CCs. It has been observed that CC apoptosis rates from morphologically abnormal oocytes were significantly higher than normal oocytes. An increase in CC apoptosis has also been associated with immaturity of oocytes and impaired fertilization. PI3K/Akt pathways is utilized by cumulus cells to support development and maturation of the oocyte. AKT1, BCL2L2 and SHC1, involved in the PI-3-K/Akt pathway, were reported to be associate with CC growth and apoptosis and could play a central role in oocyte maturation.
Study design, size, duration: This study was conducted to evaluate the correlation of cumulus AKT1, BCL2L2 and SHC1 gene expression levels with oocyte maturation. To test this hypothesis we collected CCs at different oocyte maturation profile (6 h, 12 h, 15 h, 21 h) for a total of 30 cumuli for each condition. Gene expression levels in cumulus cells were assessed using quantitative real-time polymerase chain reaction.
Participants/materials, setting, methods: CD1 mice aged 24–30 weeks were stimulated by PMSG followed by hCG 48 h later. Cumulus-oocyte complexes were collected at different after hCG (6 h, 12 h, 15 h, 21 h), for a total of 30 cumuli for each condition. Total RNA was extracted from CCs using the Arcturus Pico Pure RNA Isolation Kit. A Taq-Man Real Time approach was used to analyze gene expression on the selected transcripts.
Main results and the role of chance: We measured the gene expression levels of AKT1, BCL2L2 and SHC1 in 4 pools containing each 30 cumulus cells. Statistical analyses were performed to evaluate the correlations between CCs gene expression and oocyte maturation status. All the analyzed transcripts resulted significantly higher in CCs associated to non-mature or old oocytes (6 h and 21 h, respectively) when compared to expression levels in CCs associated to oocyte with correct timing of maturation (12–15 h). These results are in line with

our unpublished data, showing a reduced expression levels of AKT1, BCL2L2 and SHC1 in human CCs associated to positive IVF outcome. These data support the idea that CCs gene expression is strictly related to oocyte competence and could represent potential non-invasive biomarkers of oocyte quality.
Limitations, reasons for caution: Lack of information about oocyte morphometric parameters.
Wider implications of the findings: The expression of AKT1, BCL2L2 and SHC1 in CCs could represent non-invasive biomarkers to assess oocyte competence. These findings could be useful to unravel the molecular basis of cumulus-oocyte complex correct development.
Trial registration number: Not required.

P-267 Comparison between gene expression of in vivo or in vitro matured bovine oocytes and impact of vitrification in this expression

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Study question: Influence of In vitro maturation (IVM) and vitrification processes in the expression of genes related to oocyte competence, in a bovine experimental model.
Summary answer: IVM and vitrification techniques showed reduced levels of gene expression in oocytes. The impact was more significant after vitrification, possibly due to degradation of mRNA.
What is known already: IVM and cryopreservation of oocytes have been applied for fertility preservation, however the reproductive results are still limited, probably secondary to partial damages in the oocyte. The role of IVM and vitrification techniques in the RNA levels of genes related to oocytes structure, viability and/or function is not completely elucidated. Modifications in stocks of the specific mRNA these oocytes after IVM and vitrification may be helpful in elucidating the mechanisms of injury when these techniques are applied. The bovine model is justified by its similarity to human's physiology and also due to the scarcity of human oocytes for research.
Study design, size, duration: It is a sectional study performed in two periods to analyze the gene expression of: A) oocytes matured in vivo recovered by Ovum Pick up (OPU) (control group 1) versus oocytes submitted to IVM (treatment group 1) and B) oocytes from IVM (control group 2) versus oocytes from IVM plus vitrification (treatment group 2). A total of 20 oocytes/group, obtained from different animals, were processed individually to obtain the gene expression level.
Participants/materials, setting, methods: The expression of 42 genes related to oocyte structure, viability and/or function from Nelore cattle (Bos indicus) was analyzed. RNA extraction, cDNA synthesis and preamplification of target genes were performed followed by quantification by real time PCR (2-ddCT). Mann-Whitney test was used to compare the gene expression between groups ($\alpha = 0.05$). The relation between gene expression and the age of the donor animal from the in vivo group was also analyzed using the Spearman correlation coefficient.
Main results and the role of chance: A. in vivo X IVM oocytes: The expression was positive for 36 genes, and RNA values were statistically different between the groups (in vivo with higher expression than IVM oocytes for all differently expressed genes). ANXA2 (growth factor and intracellular signaling) and MAPK13 genes (cell cycle and cytoskeletal organization) showed lower expression in oocytes from older cows. **B. Vitrified X non-vitrified oocytes**: ANXA2, ARL6IP6, BCAP31, BMP15, BTG1, CD97, CDC20, CETN3, CKS2, DDR1, EEF1A1, GDF9, HK1, HSPA8, MSX1, NOBOX, PLIN2, PPA1, PSEN1, RPS15, TXN, and ZP2 genes showed lower levels of expression, while ATP5A1, CLU, DNMT3, F11R, GJA, GOT1, IGF2R, MAPK13, RPSK6B, SERPINE1, TCF4, TMIGD1, ZDHHC16 genes showed similar RNA levels between groups.
Limitations, reasons for caution: The IVM oocytes were obtained from different animals, and although they were from the same race they were not pared by age.
Wider implications of the findings: Although there is no transcriptional activity in the oocyte after resumption of meiosis, the mRNA is undergoing post-transcriptional regulatory mechanisms and it can be also degraded. Our analysis may be a tool for the development of more suitable maturation and/or cryopreservation mediums to prevent the alteration of mRNA levels.
Trial registration number: no.

P-268 The cumulative pregnancy rate is higher after fresh eSET than after eDET of top quality cleavage stage embryos

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Study question: For good prognosis patients, is cleavage-stage fresh eSET an optimum strategy for reducing multiple pregnancies without a reduction in pregnancies?
Summary answer: Fresh transfer of one instead of two top-quality cleavage-stage embryos leads not only to less multiple pregnancies, but to a higher overall pregnancy rate.
What is known already: Elective single embryo transfer (eSET) reduces multiple pregnancy rates but can decrease the pregnancy rate. In good prognosis cases such as in oocyte donation, when selection is done at the blastocyst stage, eSET is considered a very reasonable approach, however, it is not widely used when transfer and cryopreservation are performed at the cleavage stage (day 2–3 embryos).
Study design, size, duration: Retrospective analysis of IVF cases with own oocytes between 2007 and 2015. Patients age <38 years, with fresh transfer of either one (eSET group) or two (eDET group) top quality embryos according to the ASEBIR classification. In all cases, at least another top quality embryo was frozen at cleavage stage.
Participants/materials, setting, methods: The mean age was 33.5 for the eSET and 34.6 for the eDET group, total top quality embryos per cycle were 3.3 and 3.6, fresh + frozen embryos were 5.9 and 5.2 respectively. In both groups 3.7 embryos per cycle were used in total, either on fresh or on frozen embryo transfers. Cumulative (fresh + frozen) pregnancy rates (gestational sac with heart activity) were compared between both groups.
Main results and the role of chance: A total of 119 fresh transfers were performed in group eSET and 108 in eDET. The pregnancy rates (FHB +ve) were 46.2% for eSET and 55.6% for eDET ($p > 0.05$), with an implantation rate of 46.2% and 37.0% $p > 0.05$) and with a multiple pregnancy rate of 1.8% and 33.3% ($p < 0.0001$) respectively. The cumulative pregnancy rate per cycle was 98.3% (117/119) in the eSET group and 88.0% (95/108) in the eDET ($p < 0.01$). Both groups of cycles were similar in terms of patient age and quality of embryos. The same number of embryos was used in both groups. The slight (non significant) increase in the implantation rate in the eSET group suggests a lack of positive cooperation for implantation between embryos.
Limitations, reasons for caution: Not being a prospective randomized trial, the patients are not randomly distributed in both groups. Although the main characteristics of the groups are similar, slightly better prognosis cases could have been allocated to the eSET group.
Wider implications of the findings: A significant increase in cumulative pregnancy rate was obtained in the eSET group. This increase, together with the decrease in the risk of multiple pregnancies, indicates the superiority of the day 2–3 eSET plus freezing approach in good prognosis cases.
Trial registration number: NA.

P-269 Vitrification of non blastulating day 5 embryos. A method to resynchronize endometrium with embryos presenting late blastocyst formation

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Study question: Can we increase the implantation potential of non blastulating day 5 embryos by expanding culture, before vitrify them as full blastocysts and replace them in a consecutive frozen embryo transfer?
Summary answer: Expanding cumulative culture time, before or/and after cryopreservation of late blastulating embryos increases their implantation rate when replaced to a resynchronized endometrium.
What is known already: Although embryos developing to full blastocysts on Day5 vs Day6 present similar aneuploidy rates, they have different implantation

potential when transferred in fresh cycles, favoring those embryos that develop faster. On the other hand when replaced in frozen cycles, day 5 and day 6 blastocysts present comparable implantation competence. Additionally, non blastulating day 5 embryos, have very low implantation rates compared to full blastocysts when they are replaced during a fresh, stimulated cycle.

Study design, size, duration: A two year, retrospective, observational study including 73 fresh and 125 frozen cycles that no blastulating embryo was observed after 120 h of culture. In all fresh cycles (group1, n = 73), the replaced embryos, presented no blastocoel formation. In the vitrified/warmed cycles, during their fresh cycle, non blastulating day 5 embryos where either cultured and vitrified on day 6 (group2, $n = 56$) or vitrified on day 5 and further cultured for 24 h post warming (group3, $n = 79$).

Participants/materials, setting, methods: The study included infertility cases of various etiologies, from two private ART settings of the same clinic, where similar protocols of controlled ovarian hyperstimulation were applied. Severe male factor cases were excluded. The mean age of women was 36.8 years. Where applicable, embryos were vitrified using the FertiPro vitrification kit combined with a closed carrier system (VitriSafe).

Main results and the role of chance: Clinical pregnancy rate was the main outcome and was compared between Group 1 vs Group 2 and Group1 vs Group 3. Statistically significant differences were observed in the clinical pregnancy rate of fresh transfers of non blastulating embryos in Group 1 vs transferring day 6 vitrified-warmed blastocysts in Group 2 (27.4% vs 53.7%, $p = 0.002$) and respectively for transferring vitrified morulae on day 5, which were further cultured for 24 h post warming, in Group 3 (27.4% vs 55.1% $p = 0.001$). Extending cumulative culture duration, before or after cryopreservation, giving more time to fully compacted day 5 embryos to develop to full blastocysts, while replacing them to a resynchronized, day 5 progesterone endometrium, increases the implantation potential of such embryos.

Limitations, reasons for caution: Allocation of cases to the fresh or the vitrified-warmed embryo transfer groups was based on couple's acceptance to cancel the fresh transfer, on the workload of the lab and finally on the day of the week but in general it cannot be considered as a random process.

Wider implications of the findings: Findings seem to agree with the small amount of published data. Further larger studies need to be conducted to verify whether cryopreservation of embryos should be performed only at a predefined developmental stage, appropriate for the endometrial synchronization.

Trial registration number: n/a.

P-270 In vitro coculture system of autologous endometrial cells and human early embryo development: randomized study update

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Study question: What is the influence of an autologous endometrial cell co-culture system on the rate of top quality blastocysts compared with conventional culture medium in IVF?

Summary answer: Autologous endometrial cell co-culture significantly improved the rate of top quality blastocysts (TQB) compared with conventional culture medium.

What is known already: In vitro culture conditions, including culture medium, affect early embryo quality. Autologous endometrial co-culture (ECC) using the patient's own endometrial cells (EC) has been reported in order to mimic the microenvironment of early embryo development under IVF conditions.

Study design, size, duration: This interventional, monocentric, randomized, double-blind controlled trial was conducted at our clinic from April 2013 to March 2015 and is still ongoing. Eighty-five IVF couples were enrolled into the study: 48 patients cultured in conventional culture medium (control group) and 37 patients using an autologous co-culture system of EC (study group).

Participants/materials, setting, methods: For each patient, an endometrial biopsy was performed during the luteal phase of the cycle prior to ongoing IVF. For the co-culture group, EC were isolated from biopsies and cultured from the day after ovulation triggering. At day 2, embryos were placed either on ECC or in conventional culture medium according to patient randomisation. The results confirm an increase in TQB for a similar blastulation rate.

Main results and the role of chance: At day 2, a total of 261 and 217 top quality embryos were obtained in the control and co-culture group (mean ± SD per patient 5.4 ± 2.4 and 5.9 ± 2.1 respectively). The blastulation rate was 65.5% (171/261) versus 64.1% (139/217) in the control and co-culture groups respectively. Considering blastocyst quality, the proportion of usable blastocysts (for fresh replacement or cryopreservation) in the co-culture group was significantly higher (77.0%) compared to the control group (62.6%) ($p < 0.05$). In single patient comparison (control group randomized patient coming back for co-culture), the rate of TQB (D5/6) using co-culture was 43.5% compared to 18.9% in conventional culture medium ($p < 0.05$).

Limitations, reasons for caution: N/A.

Wider implications of the findings: Based on these data the use of a co-culture system of EC could potentially lead to the need for less IVF cycles per patient by increasing the number of available embryos per cycle. This hypothesis remains to be demonstrated by further analysis.

Trial registration number: NCT01886118.

P-271 Blastomere nuclear fading morphokinetic: a new perspective of early embryo biology

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Study question: Is it possible to obtain individual blastomere cell cycle information taking into consideration its nuclear fading as a time-point?

Summary answer: Determination of blastomere nuclear fading morphokinetics indicates an elongation of interphases and shortage of mitosis across cell divisions up to 8-cell stage.

What is known already: Time-lapse systems have been widely introduced in clinical routine, however there is no common consent on improvements on embryo selection and pregnancy rates. In addition, sibling embryos have a cell division pattern close to each other independently from its quality. Related to human embryonic genome activation, some studies indicate that embryo starts transcription as early as 2-cell stage. Also, first mitosis requires organization and polymerization of the mitotic spindle for the first time. Recent studies have shown correlation between morphokinetics and embryo genome activation, but none of them have considered the duration of mitosis and interphases across early embryo development.

Study design, size, duration: Retrospective clinical study. A total of 185 embryos from 113 IVF/ICSI cycles performed between January and December 2015. Embryos were cultured and analyzed morphokinetically. From 185 embryos, 124 were transferred. Only embryos with 100% ($n = 28$) or 0% ($n = 96$) of implantation were included to analyze. In addition, embryos that reached blastocyst stage were also analyzed ($n = 60$ from those transferred and $n = 61$ from those non-transferred, but frozen).

Participants/materials, setting, methods: Embryos were cultured in single step culture media (Irvine®) in a benchtop incubator with time-lapse system (Miri-TL. Esco systems®). Morphokinetic data from embryos were used to calculate interval parameters (cc2, s2, cc3 and s3). Regarding blastomeres, its nuclear fadings were used to calculate interphases and mitosis at every stage from 2 to 8 cells. Inter-parameter and inter-group comparisons were tested for normal distribution and statistically evaluated using non-parametric *U*-Mann Whitney test.

Main results and the role of chance: Comprehensive time-lapse analysis for interval parameters regarding blastomere dynamic (cc2, s2, cc3 and s3) showed a significant difference for cc3 between "Blastocyst reaching embryos" and "Not implanted" (13.67 ± 4.45 vs 13.01 ± 5.57 respectively). Intra-group analysis of interphases from blastomeres at 4-cell stage compared to interphases of respective progenitor cells (at 2-cell stage) were significantly larger, in all cases for the three different groups of embryos. Inter-group interphases analysis of parameters did not show significant differences only for just one blastomere interphase at 4-cell stage between "Blastocysts reaching embryos" and "Not implanted" (13.43 ± 3.2 vs 12.07 ± 2.67 respectively). Intra-group analysis of mitosis showed that first mitosis was significantly larger than second mitosis in all groups of embryos; Second round of mitosis was significantly larger than third round of mitosis in "Not implanted" and "Blastocyst reaching embryos" but not in the "implanted" group. Inter-group analysis of mitosis did not show any difference between groups.

Limitations, reasons for caution: No embryo quality was taken into consideration for embryo group stratification. Impaired endometrial receptivity could have drawn potentially good embryos to the "Not implanted" group. Sample size limits the study to non-parametric analysis.
Wider implications of the findings: This new parameters are reflecting biological events of early development. First mitosis is significantly longer than the following ones. This could be due to higher complexity in first spindle organization. Interphase duration seems to be longer in daughter cells, this could be explained as a result of embryonic genome activation.
Trial registration number: 0.

POSTER VIEWING SESSION

ENDOMETRIOSIS, ENDOMETRIUM, IMPLANTATION AND FALLOPIAN TUBE

P-272 Syndecan-4 expression is upregulated in endometriosis and contributes to an invasive phenotype

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Study question: Does altered expression of the transmembrane proteoglycan Syndecan-4 contribute to the pathogenesis of endometriosis?
Summary answer: Upregulation of Syndecan-4 in endometriosis may facilitate establishment of ectopic lesions by promoting invasive growth via Rac1, ATF2 and MMP3.
What is known already: Syndecan-4 is a regulator of cell motility and invasiveness in fibroblasts and in malignant diseases. Endometrial Syndecan-4 mRNA expression varies in a menstrual-cycle-dependent manner, suggesting hormonal regulation.
Study design, size, duration: Histopathological investigation of eutopic endometrium and experimental laboratory study on an endometriotic cell line (12Z). For the immunohistological investigation of Syndecan-4-expression, eutopic endometrium of 106 women (62 controls/44 endometriosis) from the IVF centre of Münster University Hospital aged 23–44 undergoing Pipelle Biopsy and diagnostic exploratory laparoscopy were studied (Ethical appoval number 1 IX Greb). The human endometriotic cell line 12Z was transiently transfected with Syndecan-4 small interfering RNA and investigated for changes in cell behaviour.
Participants/materials, setting, methods: Syndecan-4 expression in eutopic endometrium was evaluated immunohistochemically in endometrial glands and stroma. Scoring results were correlated with the stages of the menstrual cycle and presence or absence of endometriosis. Quantitative polymerase chain reaction was used to measure Syndecan-4-dependent expression changes of MMP2, MMP3, MMP9, Rac1 and ATF2. Altered cell behavior was monitored by Matrigel invasion assays and cell viability assays.
Main results and the role of chance: Syndecan-4 expression was significantly higher in the glands and stroma of endometriosis patients compared to controls ($p < 0.001$), whereas no menstrual-cycle dependent expression was observed. In 12Z cells, Syndecan-4 depletion did not affect cell viability, but resulted in a significantly reduced matrigel invasiveness ($p < 0.05$), and reduced expression of the small GTPase Rac1, the transcription factor ATF-2, and MMP3 ($p < 0.05$).
Limitations, reasons for caution: The patient collective investigated is derived from an IVF centre, which may have introduced a bias. Only eutopic endometrium, but no ectopic lesions have been studied. The functional in vitro data have been generated on an immortalized cell line, which may partially differ from results generated in primary cells.
Wider implications of the findings: The upregulation of Syndecan-4 in the eutopic endometrium of endometriosis patients may facilitate the pathogenetic process by promoting invasive cell growth via Rac1, MMP3 and ATF-2.
Trial registration number: Not applicable.

P-273 The use of resveratrol for pain in endometriosis – a randomized clinical trial

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Study question: Compared to placebo, does resveratrol (40 mg/day) reduce pain scores after 42 days of use in women with endometriosis?
Summary answer: In women with endometriosis, pain scores after 42 days of daily use of 40 mg of resveratrol are not significantly different from placebo.
What is known already: Pelvic pain is the major complaint among patients with endometriosis. An open label, not randomised trial, has shown that resveratrol reduces up to 90% pain levels of women with endometriosis, compared to placebo.
Study design, size, duration: This randomised, double blinded, placebo controlled trial enrolled 44 subjects. Allocation sequence was concealed in coded sequenced opaque sealed envelopes. Sample size was calculated to have a 95% chance of detecting, as significant at the 1% level, a 90% reduction comparing placebo and resveratrol in a 0 to 10 pain scale (i.e., a final pain score of 3.5 in the placebo group to 0.5 in the resveratrol group).
Participants/materials, setting, methods: Women between 18–50 years old with laparoscopic diagnosis of endometriosis were eligible for the study. All subjects received an COC for 42 days, to be taken with 42 identical capsules containing 40 mg of resveratrol or placebo in coded bottles. Pain scores were measured using an analog visual scale (AVS) on day 42. Side effects and use of additional pain medication was recorded. Compliance was verified by inspection of returned blisters and bottles.
Main results and the role of chance: Mean (95%CI) pain scores at day 0 were 5.4 (4.2 to 6.6) and 5.7 (4.8 to 6.6) in placebo and resveratrol groups. After 42 days of treatment, mean pain values were [3.5 (2.2 to 4.9); $n = 22$] and [2.9(1.8 to 4); $n = 22$] in the placebo and in the resveratrol groups, respectively ($p = 0.7$–2-way ANOVA with repeated measurement -2WA-RM). However, a significant reduction in pain levels was found between day 0 and day 42, in placebo ($p = 0.01$ -2WA-RM) and in the resveratrol group ($p = 0.0007$ -2WA-RM
Limitations, reasons for caution: These results were limited to the use of resveratrol at this dosage and for 42 days of use.
Wider implications of the findings: Pain difference between both groups after 42 days of treatment was 18%, a lower reduction compared to previous study. Such reduction casts doubt on its clinical significance and the need to seek further research.
Trial registration number: ClinicalTrials.gov, number NCT02475564.

P-274 The prevalence of high risk human papillomavirus in ovarian endometriosis

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Study question: To investigate whether human papilloma viruses (HPV) are associated with ovarian endometriosis lesions.
Summary answer: Findings indicated a higher rate of high risk HPV infection among patients with endometriosis but it was not associated with age, marital status, and parity.
What is known already: Endometriosis affects approximately one in ten women of reproductive age. The exact pathogenesis of the disease has not been known clearly. One of the main reasons is retrograde menstruation of endometrial cells into the peritoneal cavity. It is also suggested that intrauterine infection could initiate the endometriosis process by activating pro-inflammatory pathways and innate immunity. Beside the role of HPV in the pathogenesis of many malignant and non malignant diseases has been reported. The concept of viral infection as origin of endometriosis developed recently. It seems necessary to determine the prevalence of HPV infection in ovarian endometriosis.
Study design, size, duration: Tissue sections of 50 ovarian endometriosis and 49 ovaries without endometriosis (control group) were selected. The mean age in the case and control groups was 43.02 ± 7.64 and 42.24 ± 13.13,

respectively. Samples of ovarian endometriosis were obtained by laparoscopic surgery of the involved patients and control samples were from ovarian tissue of women of reproductive age undergone ovarian surgery for reasons other than endometriosis.
Participants/materials, setting, methods: Tissue sections of paraffin embedded samples were deparaffinized with xylene and rehydrated by graded ethanol and Double Distilled Water (DDW). HPV DNA detection was performed using HPV Screening PCR Kit. High risk HPV was detected using HPV High Risk Typing PCR Kit. The kit was used for a multiplex in vitro nucleic amplification test for genotyping of HPV types 16, 18, 31, 33, 35, 39, 45, 52, 56, 58 and 59 in clinical specimens.
Main results and the role of chance: Samples with and without endometriosis were not different significantly regarding age ($p = 0.71$), marital status ($p = 0.05$) and parity ($p = 0.46$). High risk HPV infection was detected in 42(84%) and 27(55.1%) of samples with and without endometriosis, respectively ($p = 0.02$, X2 = 8.32). Mean of age and parity was not significantly different in subjects with and without HPV infection in both studied groups ($p = 0.7$ and $p = 0.06$ for age in case and control groups, respectively and $p = 0.32$ and $p = 0.09$ for parity in case and control groups, respectively).
Limitations, reasons for caution: In this study we have no accessibility to record more detailed information from studied patients. The high rate observed in both groups may be due to errors of laboratory measurements and other environmental, genetic, or ethnic background of studied population which should be investigated in our future studies.
Wider implications of the findings: HPV may increase the capacity for invasiveness of the endometrial cells into the peritoneal cavity along with retrograde menstruation. Therefore, the findings could provide us baseline information regarding the pathogenesis of endometriosis and the role of viral infection and their impact on future cancer development in this group of patients.
Trial registration number: research project number 391374.

P-275 MK2206 and U0126 synergistically inhibit proliferation of deep endometriotic stromal cells and autophagy inhibition enhances the therapeutic effects of the combination therapy in vitro

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Study question: Is co-targeting the serine/threonine kinase AKT and extracellular regulated kinase (ERK) signaling pathways effective treatment in deep infiltrating endometriosis (DIE)?
Summary answer: MK2206 (an AKT inhibitor) and U0126 (a MEK inhibitor) synergistically inhibit proliferation of deep endometriotic stromal cells (DES) and autophagy inhibition enhances the effects.
What is known already: We previously showed that the serine/threonine kinase AKT and ERK signaling pathways may cooperate to support growth of DIE by enhancing cell proliferation and survival of DES in vitro. In addition, we showed that increased matrix stiffness promoted cell proliferation of DES in vitro. Studies showed that matrix stiffness affects responsiveness to cytotoxic drugs in a cell-dependent manner. Current drug screening assays are performed in rigid plastic, which is much stiffer than that occurring in vivo. To investigate cell responses to drugs, it is critical to model in vivo tissue compliance conditions in vitro.
Study design, size, duration: For this laboratory study, endometrial and/or endometriotic tissues from 46 patients who had histological evidence of DIE and endometrial samples from 24 patients without endometriosis were analyzed.
Participants/materials, setting, methods: We evaluated the effects of MK2206 and U0126 on inhibition of cell proliferation, apoptosis, cellular senescence, autophagy and the actin cytoskeleton of DES and endometrial stromal cells of patients with (EES) or without (NEES) endometriosis on polyacrylamide gel substrates of varying stiffness (2-, or 30 kilopascal (kPa)) or plastic. Synergism was determined by calculation of the combination index (CI) according to the median-effect method of Chou and Talalay using the CompuSyn software.
Main results and the role of chance: MK2206 and U0126 synergistically (CI < 1) inhibit proliferation of DES, but antagonistically inhibit that of EES and NEES. U0126 induced significantly higher percentages of Annexin V-positive cells than MK2206 ($p < 0.003$) and the combination of MK2206 and U0126 ($p < 0.04$) in DES. The combination therapy induced significantly higher percentages of Annexin V-positive cells in DES compared with EES derived from the proliferative ($p < 0.002$) and secretory ($p < 0.03$) phases. Expression of beta galactosidase and LC3A/B were induced in MK-2206 treated cells. The combination therapy induced a significantly higher inhibition of cell proliferation in EES compared with DES, when grown on 30-kPa (<0.03) or plastic (<0.03). Recovery rate from cell proliferation inhibition after the combination therapy was significantly higher in DES compared with EES, when grown on 30-kPa (<0.03) or plastic (<0.03). There was no significant difference in either inhibition or recovery of cell proliferation rate between DES and EES grown on 2-kPa after the combination therapy. Autophagy inhibitors enhanced inhibition of cell proliferation induced by the combination therapy, and more importantly, significantly decreased recovery rate in DES, when compared to cells grown on a substrate of the same stiffness (2- or 30-kPa, or plastic, with versus without autophagy inhibitor, $p < 0.05$).
Limitations, reasons for caution: Further animal experiments are required to evaluate in vivo efficacy and toxicity of the combined therapy of MK2206 and U0126 with autophagy inhibitor in endometriosis.
Wider implications of the findings: Recurrent rate is high after medical treatment with or without surgery in patients with endometriosis. The present findings suggested that the combination therapy of MK2206 and U0126 with autophagy inhibitor may be effective for treatment and prevention of recurrences in patients with endometriosis.
Trial registration number: N.A.

P-276 A meta-analysis of impact of endometrial cavity fluid on assisted reproductive technology outcomes

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Study question: To evaluate the impact of the presence of endometrial cavity fluid (ECF) on the outcome of ART cycles.
Summary answer: CPR is significantly lower in patients with ECF than that without ECF. The factors include etiological factor, the development time and the amount of ECF.
What is known already: Endometrial cavity fluid (ECF) is a fluid accumulation in the uterine cavity. The frequency of ECF among patients in some studies depended on the study population, ranging from 3.0%–8.2%, but there is no agreement on the impact of ECF on clinical pregnancy rate.
Study design, size, duration: Literature searches were conducted in the following databases: PubMed, China Academic Journals Full-text Database, China Doctoral and Masters' Dissertations Full-text Database. The selection criteria were study population of women undergoing ART cycles with ECF. Databases were searched for reports published before January 2015. Data were analyzed with RevMan 5.2.7 version. The odds ratio with a 95% confidence interval was calculated using the Mantel-Haenszel method. Six studies involving 4255 cycles were included in the meta-analysis.
Participants/materials, setting, methods: Search strategy: Literature searches were conducted in the following databases: PubMed, China Academic Journals Full-text Database, China Doctoral and Masters' Dissertations Full-text Database.
Selection criteria: The selection criteria were study population of women undergoing ART cycles with ECF. Databases were searched for reports published before January 2015.
Main results and the role of chance: Results: (1) Pregnancy rate was significantly lower in ECF group compared with NO-ECF group ($p = 0.03$). (2) Among the patients with hydrosalpinx, pregnancy rate was significantly lower in ECF group in comparison with NO-ECF group ($p = 0.02$). (3) The percentage of tubal infertility in ECF group was significantly higher than that in NO-ECF group ($p < 0.0001$). (4) After the patients with hydrosalpinx were excluded from tubal infertility, no statistical difference in the percentage of non-hydrosalpinx tubal infertility was found between the ECF and NO-ECF group ($p = 0.69$). The main factors impacting on clinical pregnant outcome include etiological factor of infertility, the time of ECF development and the amount of ECF. In addition, it was hydrosalpinx, not tubal infertility, which was related to the development of ECF.
Limitations, reasons for caution: Some studies did not clearly report the number of implantation embryo. As a result, implantation rate in patients with ECF

could not be assessed. The sample sizes of the included studies were small, especially for the ECF cases.
Wider implications of the findings: The main factors impacting on clinical pregnant outcome include etiological factor of infertility, the time of ECF development and the amount of ECF. In addition, it was hydrosalpinx, not tubal infertility, which was related to the development of ECF.
Trial registration number: none.

P-277 MiR-125b regulates endometrial receptivity by targeting MMP26 in women undergoing IVF-ET with elevated progesterone on HCG priming day

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Study question: What is the role of miR-125b in endometrial receptivity (ER) in women undergoing IVF-ET with elevated progesterone on human chorionic gonadotrophin (HCG) priming day.
Summary answer: MiR-125b triggers ER decline through reverse regulation of MMP26 function.
What is known already: Elevated progesterone on the day of HCG administration is associated with reduced pregnancy rates. A receptive endometrium is essential for successful embryo implantation. MicroRNAs play important roles in embryo implantation and miR-125b was different-expressed in endometrium in women with elevated progesterone on the day of HCG administration.
Study design, size, duration: The expression profile of miR-125b in endometrium was clarified in human and mouse model. The target gene of miR-125b was predicted by bioinformatics programs and confirmed by dual-luciferase activity assay. The role of miR-125b in cell function and embryo implantation was examined by gain-of-function in endometrial epithelial cells (EECs).
Participants/materials, setting, methods: Human primary EECs and endometrial stromal cells (ESCs) were isolated and cultured in vitro. The expression of miR-125b was detected by real-time PCR. Western blotting and enzyme-linked immunosorbent assay were performed to examine the change of MMP26 in EECs. The effect of miR-125b on cell migration and invasion was tested by transwell assay. The role of miR-125b in embryo implantation was examined by gain-of-function in mouse model.
Main results and the role of chance: The expression of miR-125b was significantly up-regulated in EECs in women with elevated progesterone during the window of implantation, and showed a progesterone-dependent effect in vitro, whereas it was constant in human ESCs. Similarly, the expression of miR-125b was significantly up-regulated in the preimplantation period, and was down-regulated in the implantation period and post-implantation period in mouse EECs. In addition, miR-125b showed a greater decrease at implantation sites than at interimplantation sites. The luciferase report assay showed that MMP26 is a target gene of miR-125b. The expression profile of MMP26 showed an inverse relationship with miR-125b in vivo and in vitro. Overexpression of miR-125b in human EECs inhibited cell migration and invasion by down-regulating MMP26, which may restrain embryo attachment and subsequent invasion of the endometrium. Gain-of-function of miR-125b in mouse model induced a significant decrease in the number of implantation sites.
Limitations, reasons for caution: The role of knock-down of miR-125b in EECs remains to be determined. Other target genes of miR-125b need to be elucidated.
Wider implications of the findings: Our study demonstrates that miR-125b regulates ER by targeting MMP26 in women undergoing IVF-ET with elevated progesterone on HCG priming day. The findings could provide an experimental basis for better understanding of the molecular mechanisms involved in disturbance of ER, and subsequently improve the diagnosis and treatment of infertility.
Trial registration number: This is a basic research without Trial registration number.

P-278 A new innovative method, the uterine immune profiling increases the live birth rate: a non-randomized control study

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Study question: Is determination of endometrial immune biomarkers able to improve embryo implantation through better understanding and potentiation of the initial dialogue between uterus and embryo?
Summary answer: Personalization of treatment in function of the patient's immune uterine equilibrium results in a very significant increase of subsequent healthy Live-birth rates.
What is known already: Endometrial remodeling events begin before implantation and are a vital process for pregnancy, preparing future maternal immune tolerance and regulating the placentation process. Within the endometrial environment during this stage, known as "the implantation window", a very peculiar influx of immune cells occurs and nearly completely switches local immunity from the adaptive (Th1) to the innate (Th2) type. This transient immune switch, together with adequate uNK cell activation, appears fundamental in enabling the establishment of local maternal tolerance and survival of the fetus. Nevertheless, the endometrial immune equilibrium has been almost never been considered a key factor to be considered.
Study design, size, duration: Between 2012 and 2014, 193 patients (analyzed group) enrolled in our IVF program benefitted of an endometrial immune profiling. They subsequently had an effective fresh or frozen embryo transfer (ET). If an immune dysregulation have been diagnosed (over or low-endometrial immune activation), the subsequent uterine preparation was personalized accordingly. The analyzed group was paired to a control group (193 patients) according to biological criteria (see below) at the time of ET and their outcome compared.
Participants/materials, setting, methods: Each analyzed patient was paired at the time of the embryo transfer with the closest patient enrolled in our ART program (with no previous endometrial analysis) according to following criteria: same age category; if fresh ET: same method of fertilization, same category of mature oocytes, same stage of ET; if frozen-thawed ET: same stage and number of embryos transferred. Birth rates and Implantation rates were compared between analyzed and control groups.
Main results and the role of chance: While no difference was observed at 3 weeks of pregnancy (WG), implantation rates (IR) at 10 WG and at birth in the analyzed group were significantly higher than observed in the control group (19.4% and 19.4 respectively versus 12.18 and 11.6%, $p = 00.3$ and $p = 0.01$). The most impressive data was the drastic difference regarding miscarriage rate per initiated pregnancy among analyzed and control (respectively 17% versus 41%, $p = 0.003$). Consequently, the live birth rate (LBR) was significantly higher in the analyzed group compared to the control group (27.5% versus 16.6%) despite higher duration of infertility and higher range of previous IVF/ICSI attempts and number of previous ET in the analyzed group.
Among the 193 patients analyzed, 21.7% (42/193) did not have immune deregulation but 78.3% (151/193) showed deregulation of their uterine immune environment at the time of the evaluation and therefore had at their subsequent attempt of ET personalized treatment according to their profile. The birth rate in the deregulated treated group reached 30.46% compared to 16.56% in the respective non-investigated control group ($p = 0.004$). In the analyzed subgroup with no deregulation, no difference was observed. Birth rates were strictly identical between not deregulated and control patients (16.95%).
Limitations, reasons for caution: Only randomized control study may confirm such innovation as well as placebo versus treatment study for each sub-group of immune activation.
Wider implications of the findings: Endometrial immune profiling seems efficient to determine if a woman's uterus is immunologically ready to accept an embryo and, if not, the specific immune mechanisms involved. Such diagnosis may guide physicians through an effective understanding so that they provide treatment that optimizes the conditions of uterine receptivity.
Trial registration number: None.

P-279 Single-cell transcriptome analysis of endometrial tissue

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Study question: How can we study the full transcriptome of endometrial stromal and epithelial cells at the single-cell level?

Summary answer: By compiling and developing novel analytical tools for biopsy, tissue cryopreservation and disaggregation, single-cell sorting, library preparation, RNA sequencing (RNA-seq) and statistical data analysis.
What is known already: Although single-cell transcriptome analyses from various biopsied tissues have been published recently, corresponding protocols for human endometrium have not been described.
Study design, size, duration: The frozen-thawed endometrial biopsies were fluorescence-activated cell sorted (FACS) to distinguish CD13-positive stromal and CD9-positive epithelial cells and single-cell transcriptome analysis performed from biopsied tissues without culturing the cells. We studied gene transcription, applying a modern and efficient RNA-seq protocol. In parallel, endometrial stromal cells were cultured and global expression profiles were compared with uncultured cells.
Participants/materials, setting, methods: For method validation, we used two endometrial biopsies, one from mid-secretory phase (day 21, LH+8) and another from late-secretory phase (day 25). The samples underwent single-cell FACS sorting, single-cell RNA-seq library preparation and Illumina sequencing.
Main results and the role of chance: Here we present a complete pipeline for single-cell gene-expression studies, from clinical sampling to statistical data analysis. Tissue manipulation, starting from disaggregation and cell-type-specific labelling and ending with single-cell automated sorting, is managed within 90 min at low temperature to minimise changes in the gene expression profile. The single living stromal and epithelial cells were sorted using CD13- and CD9-specific antibodies, respectively. Of the 8,622 detected genes, 2,661 were more active in cultured stromal cells than in biopsy cells. In the comparison of biopsy versus cultured cells, 5,603 commonly expressed genes were detected, with 241 significantly differentially expressed genes. Of these, 231 genes were up- and 10 down-regulated in cultured cells, respectively. In addition, we performed a gene ontology analysis of the differentially expressed genes and found that these genes are mainly related to cell cycle, translational processes and metabolism.
Limitations, reasons for caution: Although epithelial cells sorting was established, the data quality per individual cell was low. This step most likely failed due to the high dose of RNases that are released by the cells' natural processes, or due to rapid turnaround time or the apoptotic conditions in freezing- or single-cell solutions.
Wider implications of the findings: The symbiosis between clinical biopsy and the sophisticated laboratory and bioinformatic protocols described here brings together clinical diagnostic needs and modern laboratory and bioinformatic solutions, enabling us to implement a precise analytical toolbox for studying the endometrial tissue even at the single-cell level.
Trial registration number: No clinical trial.

P-280 Endometrial stromal cell-derived CX3CL1 promotes CX3CR1 expression and differentiation of CD4+Foxp3+ regulatory T cell in the endometriotic milieu

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Study question: To investigate the role of chemokine CX3CL1 in the crosstalking between endometrial stromal cell (ESC) and CD4+Foxp3+ regulatory T cell (Treg) in the endometriotic milieu.
Summary answer: ESC-derived CX3CL1 promotes Treg differentiation and IL-10 and TGF-β production by activating the ERK1/2/PTEN signaling pathway.
What is known already: Endometriosis is associated with an abnormal immune response to endometrial cells, which can facilitate the implantation and proliferation of ectopic endometrial tissue. Our previous work showed that proportion of Tregs is significantly increased in the peritoneal fluid of women with endometriosis and CX3CL1 levels in peritoneal fluid are positively correlate with the progress of endometriosis.
Study design, size, duration: All of the eutopic endometrial and endometriotic tissues were obtained by laparoscopy from 59 patients with endometriosis (mean age 38.8 years). The patients had not received any GnRH analog or other hormonal drug in the 6 months prior to the surgical operation. All of the samples were obtained in the proliferative phase of the cycle, which was confirmed histologically according to established criteria. Normal endometrium was obtained from 11 disease-free women as healthy controls.
Participants/materials, setting, methods: Peritoneal fluid was aspirated from the pelvic cavity at the beginning of the standard laparoscopic procedure under general anesthesia. Flow cytometry was performed to analyze the percentage of CD4+Foxp3+ T cells, IL-10 and TGF-β levels, phosphorylation of ERK1/2, PTEN, AKT and MAPK in naïve $CD4^+$ T cells and the expression of Fas, FasL, CTLA-4, GITR, CD39 and CD73 in $CD4^+CD25^+$ Tregs, using isotypic IgG Abs as controls.
Main results and the role of chance: The supernatant from co-cultured human ESCs and macrophages not only induced Treg differentiation and increased Treg expression of CX3CR1, interleukin-10 (IL-10), transforming growth factor-β (TGF-β) and CD73 by activating the ERK1/2/PTEN signaling pathway but also repressed Treg apoptosis by downregulating Fas and FasL expression and enhanced the Treg-mediated suppression of $CD4^+CD25^-$ T cells. In addition, in vitro and in vivo trials confirmed that these effects could be inhibited by anti-CX3CL1 neutralizing Abs. The secretion of IL-10 and TGF-β by Tregs increased MMP2 expression and decreased TIMP1 expression and further stimulated the proliferation and invasion of ESCs and the growth of ectopic lesions.
Limitations, reasons for caution: Owing to the nature of the samples (collected from different patients), there may be some difference between these volunteers. Therefore, the relationship of inter-individual variability in Treg levels in the ectopic milieu is still an open question, and awaits further studies.
Wider implications of the findings: Interference with chemokine regulatory loops such as CX3CL1 may represent a novel therapeutic strategy to reduce the growth of endometriosis.
Trial registration number: None.

P-281 Risk of miscarriage in women with endometriosis: insights from IVF cycles

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Study question: To evaluate whether women with endometriosis face an increased risk of miscarriage.
Summary answer: The risk of miscarriage is not increased in women with endometriosis.
What is known already: The observation that the endometrium of women with endometriosis has peculiar characteristics inevitably raises the question of the possible impact on pregnancy outcome and, in particular, on the risk of miscarriage. However, definite conclusions cannot be drawn because the available studies are generally methodologically weak and results inevitably exposed to confounders. According to two recent independent meta-analyses using crude data from in vitro fertilization (IVF) pregnancies, the Relative Risk (RR) of miscarriage in endometriosis women was 1.31 (95%CI: 1.07–1.59) (Barbosa et al., 2014) and 1.26 (95%CI: 0.92–1.70) (Hamdan et al., 2015), respectively.
Study design, size, duration: Women achieving singleton intrauterine clinical pregnancies with the use of IVF at two Italian Infertility units were retrospectively reviewed. Three hundred thirteen women with endometriosis and 313 controls without the disease were selected between January 2008 and June 2014. Biochemical pregnancies and multiple pregnancies were excluded. The main outcome was the rate of miscarriage occurring before 12 weeks' gestation.
Participants/materials, setting, methods: Cases were women with a history of surgery for endometriosis and those who were documented the presence of ovarian endometriomas at the time of the IVF cycle. Controls were matched to cases by age (±6 months), type of cycle (fresh or frozen cycle) and study period. Exclusion criteria included abnormal uterine cavity, uterine malformations, abnormal karyotype of the woman or her partner, multiple pregnancies and clinically relevant maternal pathologies.
Main results and the role of chance: Baseline characteristics of the two study groups were mainly similar. A statistically significant difference was found for the indication to IVF (as expected), the BMI and the duration of infertility. The number of miscarriage in women with and without endometriosis was 48 (15%) and 60 (19%), respectively ($p = 0.25$). The Odds Ratio (OR) of miscarriage in affected women was 0.76 (95%CI: 0.50–1.16). The OR adjusted for BMI, parity, duration of infertility and male factor was 0.81 (95%CI: 0.53–1.25). Subgroup analyses according to the type of cycle (fresh and frozen), the number of embryos transferred, the presence of endometriomas and the history of surgery

for endometriosis did not document any subgroup at significant increased risk of miscarriage.
Limitations, reasons for caution: We do not have reliably data on the presence of adenomyosis and, thus, we could not control for this potential confounder. Moreover, not all women underwent laparoscopy and we cannot exclude some misdiagnoses. Finally, caution is needed for inferences to the more general population of natural pregnancies.
Wider implications of the findings: Women with endometriosis do not appear to face an increased risk of miscarriage. The previously detected alterations of the endometrium in affected women may be important for endometriosis development but would not affect the early development of the implanted embryos.
Trial registration number: Not applicable.

P-282 Pregnancy outcome in women with endometriosis achieving pregnancy with in vitro fertilization

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Study question: To evaluate the impact of endometriosis on obstetric complications occurring during the second and third trimester.
Summary answer: Women with endometriosis have a four-fold increased risk of placenta previa. The risk of the other obstetric complications is conversely not affected.
What is known already: There is biological evidence showing that the endometrium of women with endometriosis differs from the endometrium of healthy unaffected women. There is also cumulating evidence suggesting that complications occurring during the second and third trimester of pregnancy, such as pregnancy-related hypertensive disorders or impeded fetal growth may actually originate from local disturbances occurring at the time of implantation. On these bases, it has been repeatedly hypothesized that pregnancy outcome may be altered in women with endometriosis. However, two recent systematic reviews of the literature highlighted several methodological pitfalls of the available evidence and ultimately failed to draw definite conclusions.
Study design, size, duration: Women achieving in vitro fertilization (IVF) singleton pregnancies that progressed beyond 12 weeks' gestation at two Italian infertility units were reviewed. We recruited as cases 239 women with a history of surgery for endometriosis and/or with a sonographic diagnosis of the disease. Controls were 239 singleton IVF pregnancies without current or past evidence of endometriosis. They were matched to cases by age, type of cycle (fresh or frozen) and study period.
Participants/materials, setting, methods: A questionnaire aimed at collecting data on pregnancy and neonatal outcome and complications was routinely given to women achieving a clinical pregnancy in both involved units. This information was systematically recorded in the clinical charts and used here for data analysis. If inconsistencies emerged or if data was incomplete, charts of the obstetrical units of the two involved hospitals were also consulted and, if doubts persisted, women were contacted for clarifications.
Main results and the role of chance: Baseline characteristics of the two study groups were mainly similar. A statistically significant difference was found only for the BMI and for the duration of infertility. The majority of women with endometriosis (78%) underwent previous surgery for the disease. One-hundred eighty-seven cases and 187 controls achieved pregnancy during a fresh cycle.

We failed to observe statistically significant differences for the main pregnancy and neonatal outcomes. The rate of live birth and the incidence of hypertensive disorders, gestational diabetes, prematurity, small and large for gestational age newborns and neonatal problems did not differ. In contrast, we observed a statistically significant increase in the frequency of placenta previa in women with endometriosis (6% versus 1% in controls; $p = 0.006$). The crude Odds Ratio (OR) of placenta previa in women with the disease was 5.1 (95%CI: 1.4–17.8). The OR adjusted for BMI and duration of infertility was 4.8 (95%CI: 1.4–17.2, $p = 0.015$). All the analyses were repeated excluding women without a definite diagnosis of endometriosis (thus without a history of surgery for the disease) and results were mainly similar (data not shown).
Limitations, reasons for caution: We do not have reliably data on the presence of adenomyosis, a potential important confounder for the association with placenta previa. Moreover, not all women underwent laparoscopy and we cannot thus exclude some misdiagnoses. Finally, caution is needed for inferences to the more general population of natural pregnancies.
Wider implications of the findings: Single embryo transfer is recommended in women with endometriosis because multiple pregnancies independently increase the risk of placenta previa. Noteworthy, cesarean section for placenta previa may be particularly demanding here because of previous pelvic surgeries. On the other hand, women with endometriosis can be reassured regarding other obstetric complications.
Trial registration number: Not applicable.

P-283 Can the intrauterine infusion of hCG improve ART outcomes? Unsafe and conflicting results in meta-analyses

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Study question: Does the intrauterine infusion of hCG (IUI-hCG) prior to embryo transfer improve the ongoing pregnancy rate in patients subjected to IVF/ICSI cycles?
Summary answer: IUI-hCG infusion prior to embryo transfer does not improve the ongoing pregnancy rate after IVF/ICSI cycles.
What is known already: hCG triggers a complex signal transduction cascade that allows embryo implantation. Considering this activity, some articles have suggested that IUI-hCG increases pregnancy/implantation rates in IVF/ICSI cycles. A recent meta-analysis (Ye et al., 2015) showed that IUI-hCG infusion prior to embryo transfer increases clinical pregnancy and ongoing pregnancy rates. However, this result was based on few randomised controlled trials (RCTs), which did not include large populations. In addition, further RCT (Wirleitner et al., 2015) did not confirm this beneficial effect of IUI-hCG. Thus, the true role of IUI-hCG in IVF/ICSI outcomes still needs analysis.
Study design, size, duration: A systematic review based on electronic searches of databases up to December 2015 was conducted to identify RCTs that compared the outcomes of IVF/ICSI cycles with IUI-hCG prior to embryo transfer in the dose ≥500 UI (study group) or no injection or placebo (culture medium/control group). The primary outcome was ongoing pregnancy rate/patient. Secondary outcomes included implantation rate, clinical pregnancy rate/patient, and miscarriage rate.
Participants/materials, setting, methods: Six RCTs (2,391 patients) were included as targets for data extraction and meta-analysis. Data were combined for meta-analysis using StatsDirect statistical software. Dichotomous data were expressed as relative risk (RR) with a 95% confidence interval (CI). The measure of heterogeneity was evaluated using Cochran's Q and I^2 tests. Study data were combined using a random-effects model. p-values <0.05 were considered statistically significant.
Main results and the role of chance: Implantation rates were reported by three trials (hCG group: 29.5%, 527/1,786; Control group: 26%, 469/1,803) with no significant between-groups differences found (RR = 1.23; 95% CI = 0.80, 1.87; $p = 0.34$). Heterogeneity: $I^2 = 93.4\%$; Cochran $Q = 30.29$, $p < 0.0001$.

Data regarding clinical pregnancy rates/patient were reported by five studies (hCG group: 47.3%, 493/1,042; Control group: 40.5%, 425/1,049). Pooled analysis found that clinical pregnancy rates were significantly higher in the hCG group compared with the control group (RR = 1.31; 95% CI = 1.01, 1.70; $p = 0.04$). Heterogeneity: $I^2 = 83.6\%$; Cochran $Q = 24.36$, $p < 0.0001$.

Data regarding the rate of miscarriage were reported by four studies (hCG group: 6.0%, 59/982; Control group: 5.6%, 55/987) with no significant between-group differences found (RR = 1.07; 95% CI = 0.75, 1.54; $p = 0.69$). Heterogeneity: $I^2 = 0\%$; Cochran $Q = 2.49$, $p = 0.47$.

The rate of ongoing pregnancy/patient was reported by four studies (hCG group: 41.4%, 407/982; Control group: 36.6%, 361/987). Ongoing pregnancy rates were not significantly different in the hCG group than in the control group (RR = 1.25; 95% CI = 0.94, 1.67; $p = 0.13$). Heterogeneity $I^2 = 82.4\%$; Cochran $Q = 17.01$, $p = 0.0007$).

P-284

Abstract withdrawn by the author

P-285 Long-term treatment of uterine fibroids with ulipristal acetate improves health-related quality of life: findings from the PEARL-III (extension) randomised controlled trial

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Study question: What are the effects of repeated three-month courses of ulipristal acetate (UPA) on the quality of life in women with symptomatic uterine fibroids?
Summary answer: Significant improvements in numerous quality of life indicators were reported after UPA course 1, which were sustained after each consecutive course and at follow-up.
What is known already: Symptomatic uterine fibroids have a profoundly negative impact on the physical, psychological and social well-being of many women with this condition, and although hysterectomy provides a permanent solution to the problems caused by fibroids, it is important that women can also access long-term treatments that improve their quality of life, minimise unwanted side effects, and allow them to retain their fertility. UPA has already been shown to be an effective and safe treatment for symptomatic uterine fibroids.
Study design, size, duration: Data are from the PEARL-III extension study. This Phase III, multicentre, clinical study investigated the efficacy and safety of up to four repeated intermittent consecutive courses of 3-month open-label UPA treatment in women with symptomatic uterine fibroids. Women received UPA (10 mg, once daily), immediately followed by double-blind oral NETA (norethisterone acetate, 10 mg once daily) or matching placebo, for 10 days. Randomisation was managed in a 1:1 ratio, involving 132 women.
Participants/materials, setting, methods: Participants were pre-menopausal women with symptomatic uterine fibroids attending European clinical gynaecology centres who had completed the PEARL-III study. Quality of life was assessed using the Uterine Fibroid Specific and EuroQol-5 questionnaires at six assessments: Baseline, (pre-randomisation); End of Course 1 UPA; 10–18 days after first day of menstruation following Course 2; 10–18 days after the first day of menstruation following Course 3; End of Course 4; and Follow-up (3 months after Course 4).
Main results and the role of chance: Of the 132 women randomised, 100 were included in the final analyses (32 women did not contribute questionnaire data at more than one assessment). No significant differences in quality of life between women in the UPA+NETA group and those in the UPA+Placebo group were found and data from the two groups were pooled for analysis. Within-subjects ANOVAs showed a significant improvement in overall quality of life after UPA Course 1 which was sustained in consecutive courses up to and including Follow-up, $F(3.60, 356.61) = 88.78$, $p < 0.001$, $\eta p^2 = 0.47$, and a significant improvement in current health state after UPA Course 1, $F(3.92, 388.01) = 24.42$, $p < 0.001$, $\eta p^2 = .20$, which was also sustained in consecutive courses up to and including Follow-up. Similar benefits on other fibroid specific quality of life subscales were found.
Limitations, reasons for caution: Responses to UFS-QOL items at each Treatment Stage were made retrospectively for a three month period and thus do not discriminate between the two months of UPA treatment and the off-treatment period of one menstrual cycle preceding completion of the questionnaire.
Wider implications of the findings: Repeated intermittent courses of UPA have significant and sustained benefits on the quality of life of women with symptomatic fibroids. Long-term management of fibroids with UPA could be an important way of improving the quality of life of women with fibroids especially in those who wish to retain their fertility.
Trial registration number: PEARL III – NCT01156857 and PEARL III extension – NCT01252069.
Trial registration date: 30th November 2010.

P-286 Frozen embryo transfer (fet) in the cycle immediately after IVF/cryoall with pgd results in higher implantation rate and decreased miscarriage rate compared to deferred fet

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Study question: Is there a benefit in allowing one menstrual cycle to occur before proceeding with a FET or to directly undergo FET the immediate subsequent cycle after undergoing an IVF/PGD/cryo-all cycle?
Summary answer: Results suggest that undergoing a FET the immediate subsequent month after a cryo-all cycle leads to greater implantation success than waiting one menstrual cycle in-between.
What is known already: Refined cryopreservation techniques have displayed improved cycle outcomes after a frozen embryo transfer (FET) as compared to fresh embryo transfer (ET). Recently, several practices counsel patients to undergo a two-cycle approach: a cryo-all cycle (all viable embryos are biopsied for genomic interpretation and cryopreserved) with a subsequent FET cycle. This strategy gives practitioners a greater opportunity to monitor, influence and enhance embryo/endometrium synchrony. Presently, there is no clinical data regarding whether there is benefit to allowing one menstrual cycle to occur before proceeding with a FET or to directly undergo an FET the immediate subsequent cycle.
Study design, size, duration: Retrospective cohort analysis from June 1st, 2011 to December 31st, 2015. The study had 80% power to detect 15% difference in between groups, with an alpha error of 0.05 ($n = 150$/group).
Participants/materials, setting, methods: Patients who underwent an IVF cycle with q-PCR-based CCS in which all embryos were cryopreserved after trophectoderm biopsy were included. Cohorts were segregated into groups according to the period between biopsy/cryopreservation to the first FET: A) Immediate subsequent FET (≤45 days); B) One month in-between IVF and FET (>45 days). The study was restricted to FETs performed <90 days after the cycle's retrieval. The outcome measure was IR.
Main results and the role of chance: A total of 638 cycles were included (Group A: 428 cycles; Group B: 210). IR was statistically higher when a FET was carried out in the immediate menstrual cycle as compared to waiting one menstrual cycle (63.4% vs 53.0%, $p < 0.05$), with an embryo being 1.5 times more likely to implant (OR 1.5, 95% CI 1.1 – 2.1). The early pregnancy loss rate was statistically lower when a FET was completed in the immediate menstrual cycle as compared to waiting one menstrual cycle (18.1 vs 25.0%, $p < 0.05$), with a 33% less probability of having a miscarriage when proceeding more quickly to a FET (OR 0.7, 95% CI 0.44 – 0.99).

	Immediate subsequent FET	One month in-between IVF and FET	Stats
Cycles	408	204	NS
Age at IVF	36.2 ± 4.1	36.9 ± 4.0	NS
Age at FET	36.3 ± 4.1	37.1 ± 4.0	
BMI at IVF	22.7 ± 4.0	22.7 ± 4.3	NS
BMI at FET	22.7 ± 4.0	22.8 ± 4.4	NS
Day 3 FSH	6.0 ± 3.2	6.1 ± 3.2	NS
AMH	3.9 ± 4.6	3.3 ± 2.9	NS
Endometrial Thickness at FET	8.9 ± 1.6	9.1 ± 1.5	NS
Retrieved	17.4 ± 10.4	16.6 ± 8.2	NS
2PN count	11.3 ± 7.3	9.9 ± 5.2	NS
Biopsy count	5.8 ± 4.2	5.0 ± 3.5	NS
ET count	1.1 ± 0.3	1.2 ± 0.4	NS
Clinical Pregnancy Rate	68.9% (281/408)	59.8% (122/204)	$p < 0.05$
Implantation Rate	63.4% (303/478)	53.0% (132/249)	$p < 0.05$
Early Pregnancy Loss Rate	18.1% (74/408)	25.0% (51/204)	$p < 0.05$

Limitations, reasons for caution: Retrospective nature.
Wider implications of the findings: The study's results suggest that undergoing a FET the immediate subsequent month after a cryo-all cycle leads to greater implantation success than waiting one menstrual cycle in-between. Patients who undergo an IVF cycle with PGS need not be discouraged from pursuing a FET attempt in their immediate subsequent menstrual cycle.
Trial registration number: Not required.

P-287 Endometrial scratching for pregnancy following sexual intercourse or intrauterine insemination (IUI): a cochrane systematic review and meta-analysis

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Study question: What is the effectiveness of endometrial scratching in subfertile women who are attempting to conceive naturally or with intrauterine insemination (IUI)?
Summary answer: Endometrial scratching appears to be beneficial in couples trying to conceive from intercourse or IUI, however the quality of the available evidence is low.
What is known already: Endometrial scratching, performed by pipelle biopsy or a similar device, has been reported to increase the probability of pregnancy in women undergoing in vitro fertilisation (IVF), specifically in women with recurrent implantation failure (RIF). The action of taking the endometrial biopsy is believed to elicit a favourable inflammation ('scratch') within the endometrium thereby making it more receptive to an implanting embryo.
Study design, size, duration: Meta-analysis and systematic review of randomised controlled trials (RCTs) evaluating endometrial scratching in women planning to undergo IUI or attempting to conceive spontaneously (with or without ovulation induction), compared to no intervention, a mock intervention, or endometrial scratching performed at a different time or to a higher/lower degree. The primary outcomes were live birth/ongoing pregnancy and pain. Last search performed October 2015.
Participants/materials, setting, methods: A thorough search for trials was performed including electronic searches in CENTRAL, MEDLINE, EMBASE, PsycINFO, CINAHL, Google, clinicaltrials.gov and hand searching of conference proceedings. Twenty one full-text articles were screened for eligibility and 8 were eligible and included in the review. Two authors independently screened the studies, extracted data and assessed study quality. Most participants in the trials had unexplained infertility and were attempting to conceive from stimulated IUI cycles.
Main results and the role of chance: Endometrial scratching increased the probability of live birth/ongoing pregnancy (RR 2.26, 95% CI 1.54 to 3.32) and clinical pregnancy (RR 1.92, 95% CI 1.44 to 2.55) compared to no procedure or a placebo procedure. This suggests that if the chance of live birth without endometrial injury is 10%, then the probability of a live birth following endometrial injury would be between 15% and 32%. However, the results must be treated with caution as most of the included RCTs were associated with a serious risk of bias. There was no evidence of an effect on miscarriage, ectopic pregnancy, or multiple pregnancy. Pain during the pipelle procedure was reported by one study as an average of 6/10.
Limitations, reasons for caution: This review only included evidence from the RCTs, however the available studies are associated with a very high risk of bias and the evidence remains at a low or very low quality.
Wider implications of the findings: High-quality RCTs which recruit sufficient numbers of women are needed to confirm or refute these findings. Although endometrial scratching is a cheap and simple procedure which can be conducted without analgesia during a short clinic visit, it does require an internal examination which is associated with pain/discomfort.
Trial registration number: N/A.

P-288 The balance between uterine CSF3/CSF3-receptor influences fertility

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Study question: Do concentrations of CSF3 and its receptor within the uterine cavity impact female fertility?
Summary answer: Elevated uterine CSF3 in and consequent reduction of endometrial CSF3 receptor levels are associated with poor receptivity, despite beneficial effects on trophoblast invasion/migration in vitro.
What is known already: CSF3 levels in follicular fluid are demonstrated to be positively associated with embryo quality. However, clinical studies of uterine CSF3 infusion as an adjuvant in ART cycles to promote endometrial growth and implantation have had mixed results. Critically, there are no scientific studies of CSF3 within the uterine microenvironment, its impact on the endometrium or how it may influence fertility.
Study design, size, duration: All samples were collected with informed consent. Uterine lavage and tissue curettage were collected from fertile (n = early secretory, n = 15 mid-secretory) and infertile (n = 18 early secretory, n = 18 mid-secretory) women during natural menstrual cycles. Lavage and/or serum were collected at the time of oocyte retrieval from 280 women (over an 18 month period) who underwent embryo transfer in the same cycle.
Participants/materials, setting, methods: CSF3 concentrations in lavage and serum assayed using Luminex. Primary epithelial cells stimulated with estrogen and progesterone; CSF3 in media measured by Luminex, receptor concentration within harvested cells determined by western blot. Immunohistochemical analysis performed on fertile/idiopathic infertile endometrium for CSF3 receptor. Response to chronic and acute exposure of ECC1 endometrial epithelial cells (adhesion and proliferation) and HTR-8/SVneo trophoblast cells (invasion and migration) to glycosylated and non-glycosylated CSF3 monitored using Xcelligence system.
Main results and the role of chance: CSF3 was elevated in lavage of natural-cycling idiopathic infertile women versus fertile women during early secretory (p = 0.019) and mid-secretory phases (p = 0.020) of the cycle. In ART cohort, elevated CSF3, in serum and lavage, evident among women who did not become pregnant; concentration was negatively correlated with endometrial thickness. Primary epithelial cells demonstrated increased secretion of CSF3 in response to progesterone; CSF3R was down-regulated under the same conditions, suggestive of negative feedback of CSF3 on its receptor. Immunohistochemistry showed reduced or absent epithelial CSF3R in tissue from secretory phase infertile women, compared with fertile controls. Acute exposure of ECC-1 to non-glycosylated (CSF3-NG) or glycosylated (CSF3-G) CSF3 increased ECC1 cell adhesion, a similar increase was evident after chronic exposure to CSF3-NG but not with CSF3-G. Acute treatment of ECC-1 with CSF3-G or CSF3-NG also enhanced proliferation ($*p < 0.05$), with a dose response evident upon treatment with CSF3-NG. However, chronic treatment with CSF3-G demonstrated an inhibitory effect on proliferation that was not evident with CSF3-NG. Treatment of HTR-8/SVneo (trophoblast) cells with CSF3-G and CSF3-NG enhanced their migration and invasion, with a significant impact on migration mediated by CSF3-NG ($*p < 0.05$) and a significant increase in invasion observed upon treatment with both CSF3-G and CSF3-NG ($*p < 0.05$).
Limitations, reasons for caution: Both CSF3-NG and CSF3-G are recombinant, therefore not a natural human form of the protein. The use of cancer derived cell lines as opposed to primary cells must be considered. Tissue from women undergoing ART was not examined. No women in whom CSF3 was used as an adjuvant were included.
Wider implications of the findings: Improved IVF treatment outcomes for many idiopathic infertile women may be achieved by inhibiting excess CSF3 and restoration of its receptor as opposed to exposure to adjuvant CSF3 which may decrease CSF3R further. Pre-screening of idiopathic infertile women to determine CSF3 and CSF3R may improve ART success.
Trial registration number: N/A.

P-289 Factors predicting improved success with use of Gcsf in thin endometrium

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Study question: If the cases with poor endometrial vascularity and thin endometrium benefit more from Gcsf than the ones with good endometrial vascularity?
Summary answer: Using Gcsf in poor endometrial vascularity cases has significantly increased pregnancy rates compared to ones with good vascularity despite non significant increase in endometrial thickness.
What is known already: The minimum measurement of the endometrium which results in acceptable pregnancy rates is still inconclusive. Literature shows data supporting values from as low as 6 mm to as high as 9 mm. on the other hand, in some investigations, there was no correlation between the IVF outcome and endometrium thickness. A wide range of therapies have been tried and tested for improvement of endometrial thickness with non definitive conclusions. The pilot study of Gleicher et al., showed the role of G-CSF on endometrial expansion in women with unresponsive endometrium, all though they failed to prove the same in subsequent publications.
Study design, size, duration: The current study is a prospective observational study conducted at Care IVF Kolkatta during the period between January 2015 and December 2015. A total of 38 cases fitting the inclusion criterion were evaluated for the effect of gcsf on the sub endometrial vascularity, endometrial expansion and pregnancy rates. The cases served as self controls from their previous failed or cancelled cycles.
Participants/materials, setting, methods: The inclusion criteria: women aged 18–45 years, previously cancelled at least one cycle because of thin unresponsive endometrium (<6 mm) during IVF programs, previously failed IVF due to poor endometrial measurement (6–9 mm), the lack of contraindications for G-CSF treatment, no genital tuberculosis, Asherman's, fibroids, and polyps. 1 ml of 300 mcg Gcsf (Endokine™ Intas) was instilled intrauterine using Cook's IUI catheter with start of Progesterone. The end points were increased endometrial vascularity and measurement and impact on clinical pregnancy rate.
Main results and the role of chance: 38 cases fulfilling the inclusion criterion were administered intrauterine Gcsf. In one of the 38 patients embryo transfer was cancelled as the ET didn't increase beyond 5 mm. Among the 37 patients in whom transfer was done, a sub group of 17 cases had poor vascularity apart from thin ET in the first cycle. Of these 17 cases 11(11/17 = 64.7%) showed improved vascularity to Zone III & Zone IV post Gcsf administration and 8 cases of these resulted in pregnancy.(8/11 = 72.7%)(mean ET = 7.35) Of the 6 remaining cases, 3 showed improvement in vascularity compared to previous cycle and 3 didn't show any improvement.(mean ET 6.76). But none of them resulted in pregnancy. 21 patients had higher grades of vascularity in the previous cycle despite poor endometrial thickness (mean ET = 6.96) Although the mean ET increased to 7.8 mm. Post gcsf only 4 cases resulted in pregnancy (4/21 = 19%). There was no significant difference in the mean endometrial thickeness between the groups that showed improved vascularity after Gcsf administration and the cases that had higher grades of vascularity before GCSF use. (7.35 vs 7.8 $p > 0.05$). But the pregnancy rates were significantly higher in the cases that had improved endometrial vasculature (64.7% vs 19% $p < 0.01$).
Limitations, reasons for caution: The number of cases in the study is small to draw any definitive conclusions. Randomized control studies are required to evaluate this further.
Wider implications of the findings: The findings can help in identifying the subgroup of patients who would benefit significantly from gcsf administration.
Trial registration number: not applicable.

P-290 HOXA-10 and E-cadherin expression in women with recurrent implantation failure (RIF) and recurrent miscarriage (RM)

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Study question: Is the expression of HOXA-10 and E-cadherin in women with unexplained RIF and RM different to that of fertile controls?
Summary answer: HOXA-10 and E-cadherin expression is reduced in women with unexplained RIF and RM compared with fertile controls.
What is known already: Both HOXA-10 and E-cadherin play crucial roles in endometrial receptivity and increased expression in endometrium during the implantation window. However, there have been limited studies on the expression of HOXA10 and E-cadherin in women with unexplained RIF and RM.
Study design, size, duration: This was part of a prospective study undertaken within a University ART unit between August 2014 and August 2015. In total 50 women were recruited, including 12 with unexplained RIF, 20 with RM, and 18 fertile controls.
Participants/materials, setting, methods: Timed endometrial biopsy samples were obtained from women 7 days after their LH surge. The expression of HOXA-10 and E-cadherin were examined by Immunofluorescence (IF) and Immunohistochemistry (IHC). The intensity of HOXA-10 and E-cadherin expression was graded and calculated according to an H-score equation: H score = ΣPi (i+1), where I was the intensity of staining (0 = negative;1 = weak; 2 = moderate; and 3 = strong), and Pi was the percentage of cells stained at each intensity (0%–100%).
Main results and the role of chance: HOXA-10 signal was mainly localized in nuclei of stromal cells and cytoplasm of glandular epithelium cells, while E-cadherin signal was only found in the nuclei of glandular epithelium cells. In the glandular epithelium, the HOXA-10 H-scores were significantly reduced in the RIF and RM compared with the fertile controls (205.00 ± 43.85 versus 270.28 ± 65.70; $p < 0.01$ and 177.75 ± 53.69 versus 270.28 ± 65.70; $p < 0.001$, respectively). In the stroma, the HOXA-10 H scores were also significantly reduced in the RIF and RM groups (218.33 ± 46.63 versus 263.89 ± 47.95; $p < 0.05$ and 201.75 ± 51.64 versus 263.89 ± 47.95; $p < 0.001$, respectively). However, the E-cadherin H-scores were significantly reduced in the RM group (243.25 ± 53.88 versus 308.06 ± 46.18; $p < 0.001$) but not in the RIF group (272.92 ± 59.29 versus 308.06 ± 46.18; $p = 0.08$). There was a trend for a reduced expression of all these markers in the RM group compared with the RIF group, although this did not reach statistical significance. Interestingly, there was a positive correlation of H-scores between both glandular and stromal HOXA-10 and E-cadherin in all women examined ($r = 0.552$, $p < 0.001$ and $r = 0.495$, $p < 0.001$ respectively).
Limitations, reasons for caution: Although this is the first study to examine the levels of HOXA-10 and E-cadherin expression in women with different types of reproductive failure, it was not designed to correlate these levels with specific clinical outcomes.
Wider implications of the findings: HOXA-10 and E-cadherin expression is significantly reduced in women with RIF and RM compared with fertile controls. Future studies should attempt to correlate the expression of these proteins to specific clinical reproductive outcomes.
Trial registration number: NA.

P-291 Conditioned medium of endometrial stromal cells from the implantation window, but not from proliferative phase, can enhance the proliferation and migration of trophoblast stem cells

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Study question: What's the effect of conditioned medium (CM) of endometrial stromal cells (ESC; implantation window vs proliferative phase) on trophoblast stem (TS) cell proliferation and migration?
Summary answer: CM of ESC from implantation window, but not proliferative phase, contained abundant growth factors and cytokines and could significantly enhance TS cell proliferation and migration.

What is known already: During embryo implantation, the endometrium becomes receptive for embryo after sequential actions of ovarian estrogen and progesterone. In response to hormones, cytokines and growth factors secreted by the endometrium during implantation window, the blastocyst becomes activated and acquires ability to adhere to the endometrium. Precise interactions between the blastocyst and the endometrium are critical for successful embryo implantation, but largely unknown. Understanding endometrium-embryo crosstalk will help increase implantation rate in assisted reproduction technology (ART).
Study design, size, duration: We first examined the effects of CM of ESC (implantation window vs proliferation phase) on trophoblast stem cell proliferation and migration. Then we analyzed growth factors and cytokines contained in CM of ESC (implantation window vs proliferation phase).
Participants/materials, setting, methods: ESC were isolated from uterine horns of female mice on d3.5 of pregnancy, which is the implantation window, and also from those of female mice in the proliferative phase. Collected CM of ESC from implantation window and proliferative phase, respectively. MTT assay was used to determine cell proliferation. Cell migration was determined by transwell assay. Protein array was performed to compare growth factors and cytokines in CM of ESC from implantation window vs proliferation phase.
Main results and the role of chance: We found that CM of ESC from implantation window can enhance the proliferation and migration of TS cells, as assayed by MTT metabolism and transwell analyses, respectively. By contrast, CM of ESC from proliferative phase had no effect on the proliferation and migration of TS cells. Growth factor and cytokine array analyses showed that CM of ESC from implantation window contained much more vascular endothelial growth factor, granulocyte macrophage colony-stimulating factor, heparin-binding epidermal growth factor, IL-1 and IL-6, as compared with that of ESC from proliferative phase. In conclusion, we demonstrated that CM of ESC from implantation window, but not from proliferative phase, are abundant in growth factors and cytokines and can enhance the proliferation and migration of TS cells.
Limitations, reasons for caution: For better understanding endometrium-embryo crosstalk in humans, human CM of ESC and TS cells may be performed after ethical issues are settled. Mouse model of recurrent miscarriage (CBA/J♀ X DBA/2♂) may be utilized to examine whether growth factors and cytokines contained in CM of ESC could prevent recurrent abortion.
Wider implications of the findings: This study supports the concept of endometrium-embryo crosstalk and may be applied to increase implantation rate in assisted reproductive technology (ART).
Trial registration number: Not applied.

P-292 Identification of human endometrial microRNAs associated with repeated implantation failures (RIF)

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Study question: Can we identify microRNAs from endometrial biopsies involved in repeated implantation failures?
Summary answer: We identified six microRNAs differentially expressed between patients with a receptive endometrium achieving or not a pregnancy (bhCG+ versus bhCG-) after embryo replacement.
What is known already: After repeated implantation failures (RIF), it is estimated that about 1/3 of RIF are associated with abnormal endometrial receptivity. To date, the microRNAs have demonstrated their involvement in the regulation of endometrial cyclic changes during the menstrual cycle. However, only one study focused on the potential identification of microRNAs associated with RIF. Here, we reported the specific miRNAs expression profile during the implantation windows after evaluation of the endometrial receptivity status diagnosed as receptive by RT-qPCR transcriptomic approach in pregnant patients or not.
Study design, size, duration: Endometrial biopsies (n = 20) were collected during the implantation windows under hormone replacement therapy (6 to 9 days after progesterone administration). Then RNAs were extracted for mRNA and miRNA purification to perform RT-qPCR gene expression and the miRNA expression profile, respectively. The RT-qPCR gene expression consists of measuring the expression level of 13 transcripts associated to the endometrial receptivity (Window Implantation Test; Patent EP10305561.2). The miRNAs profile was evaluated with the *Affymetrix® miRNA 4.1 Array Strips*.
Participants/materials, setting, methods: Endometrial biopsies during the implantation windows were obtained from 20 patients with RIF (≥3) during IVF/ICSI cycles. At first, endometrial receptivity status was evaluated by the RT-qPCR gene expression. Then, miRNA expression profiles were evaluated in receptive patients (n = 15) with a bhCG- (n = 5) or bhCG+ (n = 10) after embryo replacement.
Main results and the role of chance: Using 3 distinct statistical analyses (Student's t test, Wilcoxon signed-rank test and ANOVA), we identified six microRNAs differentially expressed in receptive patients, with or without implantation failures. These six microRNAs were all over-expressed in receptive endometrium from patients with a negative bhCG [miR-1 (x2.5, FDR = 0.004), miR-2 (x5.9, FDR < 0.0001), miR-3 (x5.8, FDR < 0.0001), miR-4 (x6.5, FDR < 0.0001), miR-5 (x6.1, FDR < 0.0001) and miR-6 (x4.7, FDR < 0.0001)]. Two of them are members of the let-7 family that have been previously reported to play a crucial role in angiogenesis and maintain of pregnancy.
Limitations, reasons for caution: The number of samples used for miRNA analyses are low. However, the relevance of these biomarkers is being validated in independent large cohort of patients.
Wider implications of the findings: The identification of endometrial micro RNAs associated to implantation failures open new perspectives in RIF patient care management. As miRNAs can be found in the bloodstream, exploration of these biomarkers as potentially circulating miRNAs might provide non-invasive diagnostic tool, avoiding performing an endometrial biopsy, to diagnose the pregnancy outcome.
Trial registration number: Not applicable.

P-293 Day 3 embryo transfer improves clinical pregnancy rate in ART patients over 42 years of age

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Study question: What is the optimal embryo transfer policy for ART patients 43 years of age and older?
Summary answer: The pregnancy rate in age classes ≥43 years was higher in cleavage stage (D3ET) compared to that of blastocyst stage (BT) transfer.
What is known already: It is frequently documented that a woman's age is inversely correlated with her fertility and the average age of infertility patients is rising because people marry later these days in Japan.

The Japan Society of Obstetrics and Gynecology (JSOG) reported that the percentage of women undergoing ART who were 40 years of age or older had increased from 31.2% in 2007 to 33.4% in 2009.
Study design, size, duration: We carried out a thorough review of 505 cycles (data from January 2013 to March 2015), from women undergoing ART who were 43 years and older which had undergone vitrified-thawed embryo transfers in a hormone replacement cycle.

Age categories were divided into 5 groups: 43, 44, 45, 46, and ≥47 years.
Participants/materials, setting, methods: All patients were treated with a clomiphene protocol. Retrieved oocytes were fertilized by ICSI. All embryos were vitrified at pronuclear stage and warmed using Kitazato protocols as described by the manufacturer. Hormone replacement treatment was used for all ET cycles.
Main results and the role of chance: The pregnancy rates (D3ET) of each age category was 14.8% (27/182), 11.9% (13/109), 10.0% (8/80), 3.0%

(1/33), and 0% (0/21), respectively. The respective pregnancy rates of BT were 7.5% (3/40), 4.2% (1/24), 0% (0/11), and 0% (0/5). The pregnancy rates of D3ET showed the higher tendency than that of BT. The rate of spontaneous abortion (D3ET) was 40.7% (11/27), 76.9% (10/13), 75.0% (6/8), and 100% (1/1), respectively. All the pregnancies resulting from BT resulted in a miscarriage.

Limitations, reasons for caution: This was an exploratory study with a limited sample size. Further research is necessary in detail.

Wider implications of the findings: Oocyte number and quality decrease with advanced maternal age reducing the chances of a viable pregnancy after ART. It is vital to make the general population aware of the high risk, and to allow them to make their own life plan concerning pregnancy and childbirth.

Trial registration number: N/A.

P-294 Human endometrial receptivity-associated miRNAs: beyond the genes

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Study question: Beyond the genes, do miRNAs are associated to endometrial receptivity status during the expected implantation windows?

Summary answer: We identified five specific miRNAs associated to a receptive endometrium compared with non-receptive endometrium during the implantation windows.

What is known already: To date, there are few studies on miRNA expression profile using Omics technologies according to the endometrial receptivity status. In addition, majority of them compared two phases of the menstrual phase (proliferative or early-secretory phase vs mid-secretory phase). Here, we reported the specific miRNAs expression profile according to the endometrial receptivity status diagnosed by the genomic approach Win-test' as receptive or non-receptive during the implantation windows in patients undergoing IVF/ICSI.

Study design, size, duration: Endometrial biopsies were collected during the implantation windows under hormone replacement therapy (6 to 9 days after progesterone administration). Then RNAs were extracted for mRNA and miRNA purification to perform the Win-Test and the miRNA expression profile, respectively. The Win-test consists of measuring the expression level of 13 transcripts associated to the endometrial receptivity by RT-qPCR (Window Implantation Test; Patent EP10305561.2). The miRNAs profile was evaluated with the *Affymetrix® miRNA 4.1 Array Strips.*

Participants/materials, setting, methods: Endometrial biopsies were obtained from 20 patients with repeated implantation failure (≥3) during IVF/ICSI cycles. The endometrial receptivity status was determined using the Win-Test allowing to classify endometrial samples as 'receptive' or 'non-receptive'. Then, miRNA expression profiles between the two groups of patients, receptive (n = 15) and non-receptive (n = 5), were performed.

Main results and the role of chance: Using 3 distinct statistical analyses (Student's t test, Wilcoxon signed-rank test and ANOVA), we identified five miRNAs differentially expressed between receptive and non-receptive endometrium [miR-1 (x-4.9, FDR < 0.0001; miR-2 (x-2.5, FDR < 0.0001), miR-3 (x-3.6, FDR < 0.001), miR-4 (x-3.9, FDR < 0.0001), miR-5 (x-2.1, FDR < 0.0001)]. All of them were down-regulated in receptive endometrium tissues and associated with the over-expression of the 13 specific genes biomarkers of the Win-Test. These 5 miRNAs target 165 over-expressed mRNAs during the implantation windows, including the 13 specific genes biomarkers of the implantation window, that were reached to several biological functions, including the granulocyte adhesion, the epithelial adherent junction signalling, the tight junction signalling and the ILK signalling playing a crucial role during implantation.

Limitations, reasons for caution: The relevance of these miRNAs biomarkers of the endometrial receptivity in endometrial tissues obtained during the implantation windows is being validated in independent cohort of patients.

Wider implications of the findings: The identification of miRNAs in endometrial tissues associated to endometrial receptivity opens new perspectives in poor implanted patient. As miRNAs can be found in the bloodstream, exploration of these biomarkers as potentially circulating miRNAs might provide non-invasive diagnostic tool to appreciate the endometrial receptivity, and consequently, to increase pregnancy rate.

Trial registration number: Not applicable.

P-295 Endometrial thickness does not predict endometrial receptivity

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Study question: Does the endometrial thickness measured by ultrasound before progesterone administration predicts endometrial receptivity assessed by molecular analysis?

Summary answer: Endometrial thickness (ETh) before progesterone administration does not predict endometrial receptivity analyzed by molecular endometrial receptivity array (ERA) at the window of implantation (WOI).

What is known already: A number of studies have investigated the association between endometrial thickness by 2D ultrasound with endometrial receptivity in patients undergoing ART procedures, but no clear conclusions have been obtained except that an endometrial thickness between 6 and 12 mm at the day of hCG administration or prior to progesterone administration in a hormonal replacement therapy (HRT) cycle is considered as receptive.

Study design, size, duration: Retrospective study including 482 patients analyzing the association between endometrial thickness (ETh) and endometrial receptivity status assessed by ERA. ETh was measured by 2D ultrasound the day before progesterone supplementation in HRT cycles prior to ovum donation or frozen embryo transfers, ERA was performed during the WOI after 5 days of progesterone administration. Patients were classified as Eth <6 mm, 6–12 mm, or >12 mm; ERA results were considered as Receptive (R) or Non-receptive (NR).

Participants/materials, setting, methods: ETh was measured as the sum of the two endometrial layers on a longitudinal transvaginal US scan at the site of maximum thickness. For ERA analysis RNA was extracted and those with RNA integrity >7 were assessed. Hybridized microarrays were scanned and the data was extracted and presented in a GPR file analyzed by the ERA computational predictor obtaining the diagnosis as Receptive or Non-receptive (Diaz-Gimeno, et al., 2011).

Main results and the role of chance: The analysis of ERA during the WOI reveals that in endometria with ETh considered as receptive (between 6–12 mm) the ratio of R vs NR was 77.26/22.73 respectively, whereas in atrophic endometrium (<6 mm) the ratio was 42.85/57.14 (*p = 0.003 by Chi-square test). In hypertrophic endometria, the ratio was 64.86/35.13 not showing differences with the other groups.

Endometrial thickness (mm)	Receptive %	Non Receptive %	Total
<6	6/14 (42.85%)*	8/14 (57.14%)*	14
6–12	333/431 (77.26%)*	98/431 (22.73%)*	431
>12	24/37 (64.86%)	13/37 (35.13%)	37
Total	363	119	482

* p = 0.003 by Chi-square test.

Limitations, reasons for caution: The retrospective nature of the study limits the validity of the results.

Wider implications of the findings: For clinicians is interesting to know that endometrial thickness between 6–12 mm does not preclude the existence of a receptivity status, that is in the range of (75% R/25% NR). In atrophic endometrium, NR cases increase significantly, whereas hypertrophic endometrium is similar to normal endometrium in receptivity terms.
Trial registration number: Not apply.

P-296 Intrafollicular PTX3 level and CCs apoptosis rate in endometriosis IVF patients

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Study question: To determinate the association between follicular fluid (FF) pentraxin3 (PXT3) levels, cumulus cells (CCs) apoptosis rates and in vitro fertilization (IVF) outcome in endometriosis-IVF patients.
Summary answer: High levels of PXT3 have been detected in severe endometriosis and are associated to CCs apoptosis and reduction of fertilization rates and embryo development.
What is known already: Pathogenesis of endometriosis, a women disease during reproductive age, is related to complex mechanisms that involve estrogen and progesterone activity, angiogenesis, apoptosis and inflammation, but it remains still unclear. Endometriosis influences negatively oocyte quality and IVF success. PTX3 is a long pentraxin that plays a key role in female fertility as an essential structural constituent of the cumulus oophorus extracellular matrix and elevated levels of soluble PTX3 can be found in human follicular fluid. Infertility of PTX3 deficient mice is due to defects in ovulation and oocyte fertilization. The relationship between endometriosis stage and PTX3 levels is unknown.
Study design, size, duration: In this prospective study 73 women undergoing to ICSI-ET cycle were enrolled from March to October 2015. The two study groups consisted of mild endometriosis cases (n = 24; A Group) and severe endometriosis cases (n = 24; B Group) (ESHRE criteria). The control group was ICSI cases with mild/moderate male infertility (n = 25; C Group).
Participants/materials, setting, methods: Exclusion criteria: age>35 years, BMI > 27 kg/m^2, smoking status, basal FSH > 9 IU/mL, PCOS, diabetes and severe oligoasthenoteratozoospermia. The controlled ovarian hyperstimulation was performed with a long protocol and recombinant FSH. At the time of pick-up were retrieved follicular fluid and isolated CCs. The apoptotic index was performed by TUNEL TEST. The Enzywell PTX3 kit was used for PXT-3 determination. Oocyte quality, fertilization rate, embryo quality were assessed. Data were analyzed using the unpaired Student's t-test (p-value ≤ 0.05).
Main results and the role of chance: No statistically significant differences in PTX-3 level and apoptosis were observed in patient with mild endometriosis respect to control group (PTX-3:15.5 ± 10.5 vs 10.7 ± 6.4; %DFI: 18.3 ± 5.5 vs 12.7 ± 4). The PTX-3 level and CCs apoptosis was higher in severe endometriosis cases compared to control group (PTX-3: 22 ± 9.1 vs 10.7 ± 6.4;%DFI: 32.5 ± 5.5 vs 12.7 ± 4). The IVF outoumes (MII retrieved oocytes number, fertilization rate and embryo quality) were statistically significant lower in endometriosis patient compared to control group.
Limitations, reasons for caution: No evaluation of serum PTX3 level.
Wider implications of the findings: This research showed an increased PTX3 levels in follicular fluid and apoptosis rate in CCs of severe endometriosis patients. These results suggest a possible mechanism responsible of reduced oocyte competence and IVF outcome in endometriosis.
Trial registration number: No requested.

P-297 Changes in spontaneous behavior in rats with surgically induced endometriosis during different phases of the estrous cycle

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Study question: How can we measure pain in rats with surgically induced endometriosis?
Summary answer: We can measure pain by measuring active behavior, which is lost due to pain or discomfort in the rat.
What is known already: Women with endometriosis suffer from pain which may negatively influence physical activity. In rats, it is possible to surgically induce endometriosis. Surgical induction of endometriosis has been shown to cause hyperalgesia and allodynia as demonstrated by increased escape behavior during vaginal distention and reduced response latency in the hot plate test.
Study design, size, duration: Design: control versus treatment study; number of treated/controls: three groups: 7 controls, 9 sham operated rats, 14 rats in whom endometriosis was surgically induced; treatment duration: 7 days of observation (during one estrous cycle) after induction of endometriosis and recovery time after surgery; sampling procedures: continuous monitoring of behavior in a home cage observation system called the Phenotyper®. The locomotor activity and explorative behaviors are recorded on a continuous basis with an infrared sensitive camera.
Participants/materials, setting, methods: General approach: animal treatment model. Species: Female Wistar rats of 6–8 weeks of age. Methods and endpoints: surgical induction of endometriosis by autotransplantation of one uterine horne, which was cut into four pieces and transplanted to the abdominal wall and mesentery. Endpoints: Mobility (duration of active moving (in average seconds) of the rat) and rearing (the number of times a rat stands upright on its hind legs), recorded in the first hour after dark.
Main results and the role of chance: We did not observe a difference in overall mobility and rearing between animals with and without an estrous cycle. However, we did observe cycle-related differences.

With regard to mobility: In control rats, no differences were seen between the estrous and the non-estrous phase of the cycle regarding the number of seconds that the rat was actively moving. In the sham operated group, the rats were significantly more active in the estrous phase as compared to the non-estrous phase ($p < 0.05$), in contrast, in the endometriosis group the rats were significantly more active during the non-estrous phase ($p < 0.05$).

With regard to rearing: In the control group, the rats significantly reared more during the non-estrous phase compared to the estrous phase ($p < 0.05$). In the endometriosis group, we saw the same effect ($p < 0.01$). In the sham operated group, no difference in number of rearings was observed between the different phases.
Limitations, reasons for caution: A woman with endometriosis has a higher volume of endometrium in her abdominal cavity due to retrograde menstruation. In our model, endometrial autotransplantion did not result in a higher volume of endometrium but only in transplantation to an other site in the abdomen. This does not mimic the human situation.
Wider implications of the findings: We speculate that the loss of active behavior during the estrous phase in endometriosis rats is caused by pain or discomfort as a consequence of the presence of ectopic endometrial tissue in the abdominal cavity.

Our data provide the basis to develop a new non-invasive animal model for pain in endometriosis.
Trial registration number: none.

P-298 Recurrent implantation failure: association with single nucleotide polymorphisms

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Study question: Is there any genetic polymorphism involved in recurrent implantation failure (RIF) that could be used as molecular biomarker helping in diagnosis and treatment?
Summary answer: Next generation sequencing (NGS) of different genes has identified some single nucleotide polymorphisms that can be involved with implantation failure.
What is known already: It has been suggested that single gene disorders could be associated with regulation of invasion and angiogenesis in embryo implantation process. Polymorphisms in these genes could increase the susceptibility of implantation failure. Next generation sequencing allowed the identification of mutations in several hundred genes which can potentially lead to infertility. The use of this tool could help in the discovery of SNPs involved in implantation failure allowing a precise diagnosis.
Study design, size, duration: A cross-sectional study was conducted with blood samples of 48 women presenting RIF, subjected to IVF/ICSI protocols and 46 fertile women (control group).
RIF group: inclusion criteria: ≥2 failed IVF/ICSI attempts/≥ 5embryos transferred, age ≤39 years and normal karyotype; exclusion criteria: uterine defects, ultrasonographic evidence of hydrosalpinx, infections, endocrine problems, coagulation defects or thrombophilia and autoimmune defects.
Control group: volunteers with at least 1 live birth, without treatment and no history of miscarriage.
Participants/materials, setting, methods: Genomic DNA was extracted from the peripheral blood of RIF and control groups. The coding regions (exons), no translated variants in both 3′/5′ UTRs and intronic regions of selected genes were analyzed by NGS using TruSeq Custom Amplicon/ MiSeq-Systems. Raw paired-end reads were aligned to human reference genome GRCh37 using BWA v0.7.12. Reads were re-aligned around indels and quality scores re-calibrated using GATK–(v3.5-0-g36282e4). GATK's UnifiedGenotyper was used as a variant caller to detect SNVs on the full alignments.
Main results and the role of chance: Following the best practices of GATK for amplicon sequencing we found prevalent genetic variants in 8 of 36 genes studied (FSHR, ESR1, ESR2, CYP19A1, BMP15, TP63, TP73, LIF, TP53, USP7, IL6ST, VEGFA, AMH, AMHR2, IL6, INHIBIN B, FMR1, IgF1, GDF9, CYP17A1, IL13, APOE, LEP, LEPR, LHB, LHCGR, AR, IgF1R, CYP11A1, PGR, MMP9, MMP2, IL1B, MTHFR, FSHB, TNF) in RIF group and their frequencies are shown in Table 1.

Table 1 | Variants frequencies

Gene	rs	allele	Control	Control	RIF	RIF
			n	Minor allele frequency	n	Minor allele frequency
AMHR2	rs2071558	C>T	11	0.094	16	0.178
AMHR2	rs2272002	T>A	8	0.068	10	0.131
ESR1	rs9341077	T>C	5	0.059	8	0.102
ESR1	rs6914438	C>T	7	0.037	11	0.099
ESR2	rs1152580	T>G	6	0.049	10	0.130
IGF1R	rs3743262	C>T	4	0.040	9	0.092
MTHFR	rs1537514	G>C	4	0.054	9	0.097
PGR	rs11224558	C>T	7	0.062	12	0.184
PGR	rs10895053	C>T	7	0.058	12	0.173
PGR	rs11224560	C>T	7	0.060	12	0.169
PGR	rs11224561	C>T	8	0.062	16	0.221
PGR	rs11224563	C>T	8	0.094	15	0.176
PGR	rs1042838	C>A	8	0.092	15	0.173
PGR	rs1824128	G>T	9	0.073	18	0.236
TP73	rs3753211	T>C	6	0.065	14	0.159
VEGFA	rs3025040	C>T	10	0.072	14	0.162

Limitations, reasons for caution: More studies are necessary to confirm this data considering variations in different ethnic population.
Wider implications of the findings: Taking into account that these SNPs could be involved with recurrent implantation failure, the expansion of this research should be considered.
Trial registration number: Not applicable. The local ethics committee authorized this study.

P-299 Endometrial thickness is not an independent predictor of live birth in IVF: should we stop measuring it during monitoring of the cycle?

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Study question: To determine if endometrial thickness (EMT) is an independent predictor of pregnancy and live birth.
Summary answer: Though EMT is associated with live birth likelihood in uni-variate analyses, EMT is not an independent predictor, when controlling for confounders (e.g., age, ovarian response).
What is known already: A large number of studies have established an association of EMT with pregnancy likelihood, however, the majority of studies failed to test the impact of EMT among other important confounders. A large systematic review of 22 studies recently reported that a thin endometrium is related to a lower chance of pregnancy: the clinical pregnancy rate for an EMT ≤7 mm was significantly lower compared with cases with EMT > 7 mm [23.3% versus 48.1%, OR 0.42 (95% CI 0.27 –0.67)] (Kasius et al., Hum Reprod Update 2014). The systematic review called for further research to determine the real independent significance of EMT in IVF.
Study design, size, duration: This analysis collates data from two, large, randomized, GCP-standard, phase III trials (07EU/Prg06, NCT00827983; 07USA/Prg05, NCT00828191) on luteal phase support in IVF (Lockwood et al., Fertil Steril 2013; Baker et al., Hum Reprod 2014). Total sample size is 1,401 patients with complete data sets. Outcomes of interest: ongoing pregnancy rate (PR) and live birth rate (LBR) in relation to EMT on day of hCG Administration.
Participants/materials, setting, methods: Patients: 18 to 42 years, BMI <30 kg/m^2, <3 prior ART-cycles, baseline FSH <15 IU/L and E_2 <80 pg/mL, normal uterine cavity as per recent hysterosalpingogram, sonohysterogram or hysteroscopic exam and at least 3 retrieved oocytes. In the absence of statistical heterogeneity between studies, data were pooled and analysed on individual patient level, accounting for clustering of patients within trials. Uni-variate and multivariate analyses were performed. EMT was studied by percentile analysis and cut-offs in 1 mm steps.
Main results and the role of chance: The pregnancy rate was significantly lower in patient with low (25th percentile ≤9.5 mm; PR = 22.03%) and high (75th percentile > 12.04 mm; PR = 27.20%) EMT as compared to intermediate EMT (25th–75th percentile > 9.5 to ≤12.04 mm; PR = 50.77%; Cochran-Mantel- Haenszel test p = 0.021). A threshold of <9 mm EMT discriminates patients with or without ongoing pregnancy (odds ratio [<9 mm vs ≥9 mm] = 1.699, 95% CI = 1.23–2.35, p = 0.001; sensitivity = 88.9%; specificity = 17.5%; PPV = 39.0%; NPV = 72.6%). Patients with low EMT (5th percentile approx. ≤8 mm) had significantly less oocytes retrieved, while female age, cause of infertility and type of stimulation protocol did not differ between groups of EMT. In multivariate analyses, in which treatment arm and trial were considered as fixed effects and other predictors of pregnancy and live birth, respectively, were selected by a stepwise logistic regression, it was found that EMT is not an independent predictor of ongoing pregnancy and live birth, when adjusting for female age, number of oocytes retrieved and transfer difficulty (easy; moderately difficult; extremely difficult).
Limitations, reasons for caution: Women with thin endometrium are not underrepresented herein despite the patient selection in a phase III trial, since thin endometrium, defined as ≤7 mm, was present in 4.8% cases compared to 2.4% in the review of Kasius et al. (Hum Reprod Update 2014).
Wider implications of the findings: Extremes of EMT require further diagnostic work-up before IVF treatment, while EMT should be ignored in the majority of cases during routine cycle monitoring in IVF patients undergoing controlled ovarian stimulation and fresh embryo transfer.
Trial registration number: NCT00827983; NCT00828191.

P-300 The hormonal environment of endometrium is a primary factor, which increases the birthweight of singletons born after frozen-thawed embryo transfer

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Study question: Why does frozen-thawed embryo transfer (FET) lead to greater birthweights than fresh embryo transfer (fresh-ET)?
Summary answer: The primary factor affecting the birthweight was the pre-/post-ET hormonal environment of endometrium, not the stage of transferred embryos nor the frozen-thawed procedure itself.
What is known already: Several studies have shown that FET decreased the risk of low birth-weight in comparison with fresh embryo transfer. Furthermore, these studies indicate the cause of the increased birth-weight may be associated with the frozen-thawed procedure, suggesting the possibility of epigenetic errors in the embryos. It has also been reported that the birth weight of boys derived from frozen-thawed embryo transfers was significantly greater than that of girls.
Study design, size, duration: This retrospective study was conducted over the period from January 2011 to December 2014, on 2,281 singletons born at 36–41 weeks of gestation, following assisted reproductive technologies (ART) in a single facility. These singletons were born after 249 fresh-ET and 2,032 FET (ovulatory cycles: $n = 204$, and HRT cycles: $n = 1,828$). Meanwhile, during the HRT cycles, endometrium was prepared by estrogen and progesterone administration.
Participants/materials, setting, methods: In this study, all pregnancies were assisted with hormonal supplementation for luteal support. The mean birth-weight of singletons born after fresh-ET were compared with singletons born after FET. Furthermore, in FET cycles, the mean birth-weight of singletons was compared between ovulatory cycles and HRT cycles. Additionally, the duration of embryonic culture until embryo transfer and the quality of embryos were categorized into cleavage stage/blastocyst stage and high quality/low quality, respectively.
Main results and the role of chance: The mean birth-weight of singletons after FET was significantly higher when compared with fresh-ET (3108 g ± 384 g vs 3047 g ± 399 g; $p = 0.023$). In FET cycles, the birthweight in HRT cycles was significantly higher than that in ovulatory cycles (3119 g ± 391 g vs 3002 g ± 374 g; $p = 0.000046$). The ratios of male neonate were similar in each ET group and each cycle in FET (52.1% vs 55.4%; $p = 0.32$, and 51.6% vs 56.4%; $p = 0.20$, respectively). In HRT cycles, there were no differences between the birthweight of singletons born from blastocyst transfers and that from cleavage stage embryo transfers (3119 g ± 386 g vs 3120 g ± 352 g; $p = 0.99$). Similarly, the mean birthweight of singletons born from high quality cleavage stage embryos showed no marked difference from the mean birthweight of low quality cleavage stage embryos (3115 g ± 359 g vs 3125 ± 346 g; $p = 0.24$). Likewise there was no detectable difference in birthweight between high quality blastocysts and low quality blastocysts (3110 g ± 381 g vs 3134 g ± 395 g; $p = 0.86$ respectively).
Limitations, reasons for caution: As our study only focused on the point of birth, long-term study is required to assess the effect of freezing and thawing procedures and the influence of HRT on birthweight in each cycle.
Wider implications of the findings: The mean birthweight of singletons born after HRT cycles was significantly higher than that of FET ovulatory cycles, indicating that the hormonal environment of endometrium may be a primary factor in increasing the birthweight in HRT cycles, rather than the stage of the transferred embryos or the frozen-thawed procedure itself.
Trial registration number: Not applicable.

P-301 Initial trial of a uterine lavage diagnostic profile to predict outcome of embryo transfer

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Study question: Can a uterine lavage (hCG+2) biomarker diagnostic profile predict the outcome of a same cycle embryo transfer?
Summary answer: A uterine lavage biomarker panel combined to produce a prognostic algorithm correctly predicting the result of embryo transfer for 95.1% of women.
What is known already: Successful IVF requires both a quality embryo and a receptive endometrium. Currently there is no diagnostic test available to assess the quality of the maternal endometrium during an embryo transfer cycle. Previously we have identified a cohort of 6 biomarkers secreted by the endometrium into the uterine cavity at the time of receptivity. These markers had shown significant discrimination of fertile and primary idiopathic infertile women during natural cycles.
Study design, size, duration: All lavage was collected with informed consent over a 1 year period. Uterine lavage was collected at the time of oocyte retrieval from 85 women having a same cycle embryo transfer. Of these women (aged 25–45 years) 15 became pregnant (confirmed by ultrasound), with 10 resulting in a live birth. Individual biomarkers and ratios of biomarkers were analysed for discrimination of transfer outcomes. Logistic modelling was performed and performance assessed by ROC analysis.
Participants/materials, setting, methods: Lavage was assayed for Colony Stimulating factor-3 (CSF3), Interleukin-8 (IL8), Interleukin-6 (IL6), vascular endothelial growth factor (VEGF) and Interleukin-17A (IL17A) using Milliplex bead-based ELISA. C-reactive protein (CRP) was determined using Randox high sensitivity assay. Additional parameters, e.g., peak estradiol, progesterone, embryo quality, age, BMI, etiology, endometrial thickness etc. was collected. Pregnancy was confirmed by ultrasound; biochemical pregnancies ($n = 3$) are excluded from analysis.
Main results and the role of chance: Individually, Il6, IL8, VEGF and IL17A showed significant ($p < 0.05$) discrimination of embryo transfer outcome being elevated among women for whom transfer was unsuccessful. These were significantly ($p < 0.05$) positively correlated with progesterone concentration but not peak estradiol. Ratios of Il17A/CRP ($p = 0.067$) and VEGF/CRP ($p = 0.084$) and CSF3/IL17A ($p = 0.078$) showed a non-significant trend ($p = 0.078$) to be lower among the pregnancy group. None of the 6 biomarker levels were related to age, BMI parity, cycle number, aetiology or treatment type. CSF3 was significantly negatively correlated with endometrial thickness ($p = 0.023$).

In logistic regression modelling, the most significant factors in creating the prognostic algorithm were; age, P4, Il17A/CRP, endometrial thickness, BMI, peak E2, and Il6/VEGF. The algorithm correctly predicted outcome of embryo transfer in 95.1% of women, with an Area under the ROC curve of 0.996 with a significance level of $p < 0.0001$. Using a threshold of 0.378, a sensitivity of 100% (i.e., all actual pregnancies were predicted as pregnancy) with a specificity of 94% (i.e., 64/67 of women who failed to become pregnant correctly predicted as no pregnancy) is achieved, thus a PPV of 83.3 and NPV of 100 is obtained.
Limitations, reasons for caution: This is a single site study. Numbers are limited particularly with regard to successful pregnancies. A larger second test data set to evaluate the true diagnostic performance is required.
Wider implications of the findings: Potential identification of further biomarkers including those to identify specific risk factors, best therapy options and combination with embryo quality testing may provide a path to cycle monitoring and individualised therapy.
Trial registration number: Not applicable.

P-302 The effects of endometrial preparation regimen on pregnancy and neonatal outcomes following single vitrified warmed blastocyst transfer

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Study question: Does the difference in endometrial preparation regimen influence the pregnancy and neonatal outcomes following single vitrified warmed blastocyst transfer (SVBT)?
Summary answer: Endometrial preparation using letrozole improved live birth rate following SVBT, although the incidence of adverse neonatal outcomes was not increased in any endometrial preparation regimens.

What is known already: Different cycle modalities with regard to endometrial preparation for frozen-thawed embryo transfer (FET) are used: natural cycles, hormone replacement (HR) cycles and cycles stimulated with gonadotropin, clomiphene citrate or aromatase inhibitor such as letrozole. Currently, there is a little consensus on the most effective endometrial preparation regimen aiming to improve success rates following FET. In addition, there are few large-scale cohort studies on the relationship between the difference in endometrial preparation regimen and pregnancy and neonatal outcomes.

Study design, size, duration: The retrospective study period was from January 2010 to December 2013, including 13450 of SVBT cycles (8789 women, age: 36.1 ± 0.1) performed in Kato Ladies Clinic, Tokyo, Japan. Pregnancy and neonatal outcomes after SVBT in three endometrial preparation regimen: natural cycles (NC: n = 11208, 7264 women, age: 36.2 ± 0.02), HR cycles (HR: n = 876, 546 women, age: 35.7 ± 0.1) or cycles stimulated with letrozole (LET: n = 1366, 979 women, age: 35.3 ± 0.1) were compared.

Participants/materials, setting, methods: In SVBT cycles, blastocysts were vitrified and warmed using Cryotop safety kit. The primary outcomes were clinical pregnancy rate (CPR) with gestational sac at 6–7 weeks of pregnancy, ongoing pregnancy rate (OPR) with heart beat at 9 weeks of pregnancy and live birth rate (LBR) at 22 weeks of pregnancy over. Secondary outcomes were perinatal mortality and major birth defects of singleton delivered by SVBT.

Main results and the role of chance: CPR per SVBT cycles was 56.7% (6352/11208) in NC group, 57.0% (499/876) in HR group and 58.6% (801/1366) in LET group. OPR in NC, HR and LET groups were 50.3% (5641/11208), 51.0% (447/876) and 53.6% (732/1366), respectively. LBR was 44.6% (5000/11208) in NC group, 43.2% (378/876) in HR group and 48.3% (660/1366) in LET group. Significant difference was observed between NC and LET groups in CPR. LBR was significantly higher in LET group compared with NC and HR groups ($P < 0.05$). Perinatal mortality and major birth defect rates were comparable between NC, HR and LET groups (Perinatal mortality rates: 0.2% (4/4932), 0% (0/376) and 0.3% (2/650), major birth defect rates: 2.8% (139/4932), 3.2% (12/376) and 3.8% (25/650), respectively).

Limitations, reasons for caution: Despite the large number of SVBT cycles were included, this study is limited by its retrospective design. Further randomized controlled trials are required to clarify the effect of endometrial preparation methods on pregnancy and neonatal outcomes.

Wider implications of the findings: Our observation that LBR per SVBT cycles is significantly higher in LET group suggests that cycles stimulated with letrozole for endometrial preparation prior to SVBT would improve the pregnancy outcomes. Additionally, endometrial preparation regimen would not influence the incidence of adverse neonatal outcomes following SVBT.

Trial registration number: Not applicable.

P-303 The expression of angiotensin II receptors mRNA in granulosa-lutein cells in endometriosis patients

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Study question: Is the expression of angiotensin II receptors in granulosa-lutein (GL) cells altered in endometriosis patients?

Summary answer: The expression levels of angiotensin II receptors in GL cells were lower in endometriosis patients than non-endometriosis patients.

What is known already: Rennin-angiotensin system plays a key role in the regulation of blood pressure and the hydro-electrolytic balance. There were several reports that angiotensin II is present in high concentrations in follicular furuid (FF). Angiotensin II receptors can be classified angiotensin II type 1 receptor (AT1) and angiotensin II type 2 receptor (AT2) and others. AT1 has these functions such as vasoconstriction, angiogenesis and proliferation of cells. AT2 has these functions such as vasodilation, and apoptosis. AT1 and AT2 have been identified in human ovarian follicular cells.

Study design, size, duration: We investigate the concentration of angiotensin II in FF and the expression of AT1 mRNA and AT2 mRNA in granulosa-lutein (GL) cells in endometriosis patients with controlled ovarian stimulation (COS) for ART. FF samples were obtained from 50 IVF cycles (40 without endometriosis for control, and 10 with endometriosis). GL cells were obtained from 16 IVF cycles (10 without endometriosis, 6 with endometriosis). The patients with endometriosis were diagnosed by ultrasound and/or surgery.

Participants/materials, setting, methods: FF samples were obtained from 50 IVF cycles at the day of oocyte pick up. We examined the level of angiotensin II in FF by ELISA. GL cells were collected from FF. GL cells lightly centrifuged after removal of the oocyte. Cells washed in isolation medium twice, and separated from red blood cells using a 50% Percoll gradient. We examined the expression of AT1 mRNA and AT2 mRNA by RT-PCR.

Main results and the role of chance: The levels of angiotensin II were no significant difference between control and endometriosis group. The expression levels of AT1 mRNA in endometriosis was significant lower than control (p-0.0075). The expression levels of AT2 mRNA in endometriosis was significant lower than control (p-0.0075). There were positive relationship between AT1 mRNA and AT2 mRNA in all cases. We counted the number of oocytes retrieved. In control group (n = 10), total 40 oocytes were obtained including one immature oocyte (2.5%). In endometriosis group (n = 6), total 13 oocytes were obtained including 3 immature oocytes (23%).

Limitations, reasons for caution: The number of samples was small.

Wider implications of the findings: There was a report that the existence of a proper balance between proliferation and apoptosis in GL cells is indispensable to obtaining a fully competent oocyte. Thus we considered that lower expression levels of angiotensin II receptors mRNA in GL cells with endometriosis may affect decreased number of mature oocytes.

Trial registration number: Institutional Review Board (IRB) approval was obtained. Trial registration number is RK-140411-3.

P-304 The effects of integrin β3 on enzymes of the endocannabinoid system and endometrial receptivity

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Study question: What effect does integrin β3 have on the enzymes of the endocannabinoid system (NAPE-PLD and FAAH) and are any differences linked to endometrial receptivity?

Summary answer: Receptive endometrial cells increased FAAH and decreased NAPE-PLD transcript levels in response to an increase in integrin β3 activity. Non-receptive endometrium cells showed no change.

What is known already: Endogenous cannabinoid (endocannabinoid) concentrations need to be carefully titrated to ensure uterine receptivity, and this "anandamide tone" is vital for successful embryo implantation. Anandamide tone is achieved by a careful balance between the activity NAPE-PLD and FAAH. Furthermore, the expression ratio of NAPE-PLD: FAAH differs at the implantation zone and the inter-implantation zone.

Integrin β3 expression coincides with the window of implantation, and aberrant expression of integrin β3 has been linked to infertility. Integrin β3 expression has also been linked to pinopode expression, suggesting that integrin β3 might be involved in producing the preferred site of embryo-endometrial interaction.

Study design, size, duration: Human endometrial cell lines were examined: Ishikawa cells were used as a receptive endometrium model; HEC1A cells were used as a non-receptive endometrium model. *S*-nitroso-*N*-acetylpenicillamine (SNAP: a nitric oxide donor) was used to up-regulate the expression of the integrin β3 (in a dose-dependent manner). SNAP (0–2000 μM) was used for 48 h and each concentration was added to 6 wells of cells. RT-PCR for the enzyme genes measured and corrected for GAPDH.

Participants/materials, setting, methods: Ishikawa and HEC1A cells (5×10^5) were seeded into a 6-well plate and cultured in 2 ml of culture medium (37°C, 5% CO_2) for 24 h. Following this, culture medium was replaced with medium augmented with *S*-nitroso-*N*-acetylpenicillamine (SNAP, 0–2000 μM) and the cells were cultured for a further 48 h. Following this, the total cellular

RNA extracted and quantified (Taqman PCR) and the relative levels of NAPE-PLD and FAAH transcripts measured.

Main results and the role of chance: The experiments with the Ishikawa cells (receptive endometrium) showed a clear inverse and statistically significant correlation between the concentration of SNAP and the expression of NAPE-PLD when compared against the control ($p < 0.01$ for 500 and 1000 μM, $p < 0.05$ for 2000 μM; Tukey's multiple comparison test). It also showed positive correlation between the concentration of SNAP and the relative expression of FAAH expression. Cells treated with 2000 μM SNAP showed a statistically significant difference in the relative FAAH expression when compared against the control (p-0.001). In addition, cells treated with 50–500 μM of SNAP showed a statistically significant difference when compared against 2000 μM of SNAP ($p < 0.001$ for 50 μM, $p < 0.01$ for 100 and 500 μM SNAP; One-way ANOVA with Tukey's multiple comparison test).

In contrast, the experiments with the HEC1A cells (non-receptive endometrium) treated with increasing concentrations of SNAP did not show any significant relationship between in their expression of either NAPE-PLD or FAAH (One-way ANOVA; NAPE-PLD $p = 0.1862$, FAAH $p = 0.2170$).

Furthermore, the relative expression of NAPE-PLD in Ishikawa cells was twice that of HEC1A cells, and the relative expression of FAAH in Ishikawa cells was 100 times that of HEC1A cells, suggesting FAAH expression is a key regulator of implantation in the human endometrium.

Limitations, reasons for caution: The small sample size ($n = 6$ for each concentration) means further work is necessary before decisive conclusions can be made, however, the strong statistical significance is promising. Also, we have only shown relative expression of NAPE-PLD and FAAH rather than exploring their functionality.

Wider implications of the findings: This study supports the existing literature showing that integrin β3 and the endocannabinoid system have individually been implicated in human and animal endometrial receptivity, however, this is the first study that brings these two factors together and links them. This provides a new therapeutic target for improving fertility rates.

Trial registration number: N/A.

P-305 The window of endometrial receptivity might be displaced under different protocols for endometrial preparation

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Study question: Does the window of endometrial receptivity (WOI) change in the same patient under different protocols for endometrial preparation?

Summary answer: WOI might vary within the same patient under different protocols for endometrial preparation being hormone replacement therapy, and natural cycle with hCG the most consistent.

What is known already: The most used protocols to prepare the endometrium for embryo transfer are the controlled ovarian stimulated (COS) for fresh transfers, hormone replacement therapy (HRT), natural modified cycle with hCG triggering (NC-hCG), or natural cycle (NC) following spontaneous LH peak. Controversy exist regarding the superiority of each of them to optimize the WOI.

Study design, size, duration: Prospective study in which with 5 healthy volunteers with proven fertility between January 2014 and March 2015 in IVI Madrid that consecutively underwent COS, HRT, NC-hCG, or NC. Endometrial biopsy was obtained in COS 5 days after OPU day with 4 days with 200 mg/12 h vaginal progesterone, HRT after 5 days of added 400 mg/12 h of P4, NC-hCG, 7 days after hCG with 4 days of 200 mg/12 h of P4 and in NC 7 days after LH peak.

Participants/materials, setting, methods: A total of 20 endometrial biopsies were analyzed. RNA was extracted and those with RNA integrity >7 were assessed. Hybridized microarrays were scanned and the data was extracted and presented in a GPR file analyzed by the ERA computational predictor obtaining the diagnosis as Receptive or Non-receptive (Diaz-Gimeno et al., 2011). The level of progesterone in ng/ml was assessed at the day that endometrial biopsy was taken.

Main results and the role of chance: All the patients analyzed showed consistent WOI obtained by ERA diagnosis in HRT and NC-hCG cycles, being receptive in all of them at the time were endometrial biopsy was obtained. However, the WOI was displaced in 1 out of 5 patients undergoing COS cycles, and in 2 out of 5 patients in NC. Only 2 out of the 5 patients analyzed maintained the same WOI regardless of protocol used. No correlation was found between the levels of progesterone at the day of endometrial biopsy and the displacement of the WOI found under different endometrial preparation.

Limitations, reasons for caution: The size of study is limited, all of the volunteers had proven fertility, we do not know if infertile patients could have different results.

Wider implications of the findings: For clinicians is interesting to know that HRT and NC-hCG protocols are more predictable whereas COS or NC might induce a displacement of the WOI in the same patient. Embryo transfer must be performed always with the same type of endometrial preparation used for the ERA test.

Trial registration number: NCT02280798 in Clinical Trials.

P-306 Endometrial mesenchymal stem cells (E-MSC) can differentiate into endothelial cells in a direct co-culture-based *in vitro* model of ovarian endometriosis

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Study question: Are endometrial mesenchymal stem cells (E-MSC) directly involved in the angiogenic process supporting ovarian endometriotic implants?

Summary answer: The angiogenic potential of E-MSC can be observed as the acquisition of CD31 marker in a direct co-culture system with endothelial cells.

What is known already: Previous studies demonstrated that cells with properties of mesenchymal stem cells are present both in the human endometrium and in culture cells from ovarian endometriotic lesions. An attractive hypothesis is that endometrial stem cells may migrate into the peritoneal cavity and participate in the generation of endometriotic implants. This process is strictly supported by the formation of new blood vessels, which provide oxygen and nutrient supply to the ectopic tissue, suggesting the need of a proangiogenic microenvironment for the development of the endometriotic lesion.

Study design, size, duration: For this *in vitro* study E-MSC were obtained from seven patients receiving surgery for treatment of ovarian endometriosis at S. Anna Hospital in Turin from November 2013 to February 2015.

Participants/materials, setting, methods: *In vitro* formation of capillary-like structures was studied on growth factor-reduced Matrigel and compared with HUVEC cells. A co-culture system was established seeding HUVEC (GFP^+) and E-MSC (ratio of 1:1–3 × 10^5 cells/line) for 48 h. The acquisition of endothelial markers, including CD31, was evaluated by FACS analysis and immunofluorescence. In same culture conditions the population of E-MSC-$CD31^+$ was sorted and analyzed by Real-Time PCR.

Main results and the role of chance: E-MSC expressed mesenchymal markers (CD44, CD73, CD105, CD29 and CD90) at basal level, whereas endothelial markers (CD31, VEGFR1, VEGFR2, VEGFR3 and Tie2) were not expressed. In the tubes forming assay, E-MSC were equally able to create interconnections as well as HUVEC (control cell line). In the co-culture system, the interactions between HUVEC (GFP^+) and E-MSC induced an increase of +10% in CD31 expression in the GFP^- cell population observed by FACS analysis. Furthermore, immunofluorescence images showed that E-MSC, directly in contact with HUVECs, acquire the expression of CD31. Using a cell sorter we isolated $GFP^-/CD31^+$ (post co-culture) and Real-Time PCR confirmed the significant increase of CD31, VEGFA, VEGFR2 and

TIE2 gene expression ($p < 0.05$). On the other hand, the expression of mesenchymal genes such as Vimentin and N-Cadherin significantly decreased ($p < 0.05$).
Limitations, reasons for caution: Further studies have to be performed to prove the relevance of this biological phenomenon. An *in vivo* model of endometriosis could mimic the histological complexity of endometriotic tissues, giving more information about the interaction between E-MSC and endothelium.
Wider implications of the findings: E-MSC undergo endothelial differentiation, showing a clear angiogenic potential, due to a direct interaction with endothelial cells. It can be hypotesized that the formation of vessels during the early stages of the development of endometriotic implants could involve E-MSC.
Trial registration number: None.

P-307 VEGFA mRNA: a new biomarker allowing to detect chronic endometritis during the window of implantation?

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Study question: Can chronic endometritis (CE) be diagnosed based on the endometrial mRNA expression profiles during the window of implantation and what are the diagnostic criteria?
Summary answer: *VEGFA* mRNA expression (>0.250) in the mid-luteal phase is associated with CE (sensitivity 64%, specificity 89%).
What is known already: Endometrial morphology and function plays an important role in IVF. Prevalence of CE in patients with recurrent IVF failures ranges between 30% and 60%. Diagnosis of CE is based on the histological evaluation of endometrial biopsies on Day 7–10 of the menstrual cycle. The critical marker of CE is presence of plasmatic cells. At the same time, endometrial receptivity is assessed in the mid-luteal phase, and the possibility to combine these two tests would facilitate management of IVF patients. A universal marker of endometrial inflammation and receptivity, which could be used for tailored pre-conception management, has not yet been identified.
Study design, size, duration: The study had a case-control design and included 33 patients with tubal infertility. All patients underwent hysteroscopy and diagnostic curettage in the first phase of the menstrual cycle. Group 1 ($n = 15$) consisted of patients in whom plasmatic cells were identified in the endometrial tissue. Group 2 ($n = 18$) included women in whom no plasmatic cells were found. In the subsequent menstrual cycle, all women underwent endometrial biopsy during the window of implantation.
Participants/materials, setting, methods: Inclusion criteria: age <37 years; spontaneous ovulation; history of 2 unsuccessful IVF cycles. Exclusion criteria: endometrial thickness <8 mm during the window of implantation, contraindications to IVF, gynaecological conditions: uterine fibroids, endometriosis, PCOS. Expression of 39 genes in mid-luteal endometrial biopsies was assessed using RT-qPCR mRNA quantification. The genes studies belonged to the families of growth factor markers, cytokines, surface immune markers, matrix metalloproteinases, homeobox genes, cycloxygenase, estrogen and progesterone receptors, and apoptotic markers.
Main results and the role of chance: Only 4 genes showed significantly different expression levels in Group 1 and Group 2 (*VEGFA, VEGFA*$_{189}$, *IGFBP1*, *IL2*). Specifically, *VEGF*pmRNA levels in Group 1 [Me: 0.392 (0.228–0.548)] were almost 1.7-fold higher than in Group 2 ($p = 0.008$). *VEGFA*$_{189}$ mRNA levels in Group 1 [Me: 0.240 (0.196–0.430)] were 2.4-fold higher than in Group 2 ($p = 0.011$). *IGFBP1* mRNA levels [Me = 0.0003634 (0.0000245–0.0010467)] in Group 1 were 16.6-times higher than in Group 2 ($p = 0.032$). Multifactorial regression analysis allowed to select a single independent informative marker, *VEGFA,* which allowed to diagnose CE in the luteal phase. According to ROC-analysis, high levels of *VEGFA* mRNA expression were associated with presence of plasmatic cells in the first phase of the menstrual cycle. For the baseline level of expression set at 0.250, the area under curve was estimated to be 0.778 (0.612–0.943). The model sensitivity and specificity at the baseline level were 64% and 89%, respectively.
Limitations, reasons for caution: Our study was carried out in a relatively small subset of women with tubal infertility and normal ovulation. Therefore, the results obtained cannot be readily extrapolated on other groups of patients and need to be confirmed in larger trials.
Wider implications of the findings: This study opens new possibilities for simultaneous assessment of endometrial receptivity and detection of CE in the mid-luteal phase of the menstrual cycle in women starting IVF.
Trial registration number: N/A.

P-308 Role of STAT3 in endometrial regeneration in the mouse model of decellularized uterine matrix transplantation

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Study question: Are there any useful experimental models to study the process of uterine regeneration? What are fundamental mechanisms of endometrial regeneration?
Summary answer: We established a model of decellularized uterine matrix (DUM) transplantation to analyze uterine regeneration and revealed a significant role of STAT3 in endometrial epithelial regeneration.
What is known already: Human endometrium exhibits high capacity of regeneration, which renews every menstrual cycle to prepare for pregnancy. Mouse endometrium also shows rapid reconstruction after parturition to prepare for next pregnancy. The previous studies reported that a limited population of endometrial cells presents particularly high regeneration capacity, and however, the detailed mechanism of endometrial regeneration remains unknown.
Study design, size, duration: A mouse model of DUM transplantation, in which an artificial defect of a living recipient uterus was surgically complemented with a portion of DUM, a scaffold of extracellular matrix proteins containing no intact cells, was used in this study. Sites of DUM transplantation were histologically examined.
Participants/materials, setting, methods: Wild-type (WT) mice and uterine *Stat3* knockout mice (*Stat3-floxed/Pgr-Cre* mice) were used in this study. Resected donor mouse uteri were processed with sodium dodecyl sulfate to make them only extracellular matrices without any intact cells. The decellularized matrices were transplanted into artificial defects of recipient mouse uteri, and the sites of transplantation were examined by H&E staining, immunostaining, and fluorescence assay.
Main results and the role of chance: In the transplanted DUM of WT recipient mice, all the uterine layers were histologically recovered by post-transplantation day 28 (Day 28). In the regenerated uterus, normal hormone responsiveness in the periimplantation period were observed during pregnancy. Time-series observation of the regeneration process revealed that flat cells rapidly migrated onto the DUM and formed a new epithelial layer as early as on Day 1, and the layer became a normal columnar epithelium on Day 7, indicating prominent regeneration capacity in the endometrial epithelium. Stromal and myometrial regeneration occurred following epithelial regeneration. In the DUM transplantation model using ovariectomized WT mice, uterine regeneration was similarly observed, suggesting that ovarian hormones are not essential for this regeneration process. The prominent immunoreactivity of phosphorylated STAT3 (pSTAT3) was observed in the epithelium of WT recipients around the DUM on Day 1. Interestingly, cell proliferation and reconstruction of the epithelium at the DUM on Day 1 were markedly suppressed in uterine *Stat3* knockout mice. In addition, the human endometrium showed the heightened immunoreactivity of pSTAT3 at the menstrual period when endometrial regeneration remarkably occurs. These findings suggest a key role of STAT3 in endometrial epithelial regeneration as the initial step of the uterine/endometrial regeneration process.
Limitations, reasons for caution: We performed all the analyses using a mouse *in-vivo* model. Further investigations are required for the

establishment of an *in-vitro* uterine regeneration model using human DUM and cells.
Wider implications of the findings: DUM transplantation can be a novel approach for uterine regenerative research and medicine. This technology may clarify molecular mechanisms of uterine regeneration including STAT3 signaling, and lead to clinical application against uterine diseases such as implantation failure in the future.
Trial registration number: N/A.

P-309 Elevated nerve growth factor and its receptors levels in endometriotic tissues are associated with deep dyspareunia

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Abstract withdrawn by the authors

P-310 Interval double transfer undergoing frozen-thawed transfer cycle improves treatment success in patients with over twice consecutive IVF failures

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Study question: This prospective control study was to examine whether sequential embryo transfer improves IVF/ET success rates in patients which over twice consecutive IVF failures.
Summary answer: Interval double transfer undergoing frozen-thawed transfer cycle improves treatment success in patients with over twice consecutive IVF failures.
What is known already: It is supposed that improvement of IVF/ET outcome in women who underment endometrial biopsy prior to the recent cycle and blastocyst transfer.
Study design, size, duration: Women undergoing frozen-thawed embryo transfer with minimum of two IVF/ET failures (n = 150) were selected for our analysis. The patient selection criteria in the study were: (i) age ǧ40, (ii) normal thrombophilia screening, (iii) normal karyotype, (iv) normal endometrial by hysteroscopy investigation, (v) at least three good-quality embryos that were frozen on day 3 of culture. The study was carried out from April 2013 to November 2014.
Participants/materials, setting, methods: Patients undergoing frozen-thawed embryo transfer were prospective by randomized to one of three groups according to a randomized controlled table. Group A sequential transfer on one day 3 embryo followed by a 5 day blastocyst. Group B underwent mock transfer on day 3 and two blastocyst on day 5. Group C just transfer two embryos which had developed to blastocysts after being preserved in liquid nitogen and thawed. Clinic pregnancy rate (PR) was measured.
Main results and the role of chance: The clinical pregnancy rate of Group A was highest of three groups (77.5% vs. 58.7% vs. 59.6% p = 0.03) while the clinical pregnancy rates of Group B and Group C were not significantly different (58.7% vs. 59.6%).
Limitations, reasons for caution: A statistic difference may be reached only after we increase the sample size.
Wider implications of the findings: interval double transfer may improve the clinical pregnancy rates for patients with consecutive IVF/ET failures compared with regular 5 day blastocyst transfer. This study has shown that co-culture of early-stage embryos with endometrial epithelium may increase success rate of IVF/ET, mechanical stimulation of the endometrial dose not play a role.
Trial registration number: Guo H, Li Y, Luo K, Gong F.

P-311 The aerobic glycolysis induced by HIF1α-prohibitin pathway in Endometriosis

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Study question: Whether HIF-1a can regulate the expression of PHB in EMs.
Summary answer: HIF-1α may combine with the HER in PHB to down regulate the expression of PHB in EMs.
What is known already: Prohibitin (PHB) combined with hypoxia inducible factor-1α (HIF-1α) has been confirmed to induce the aerobic glycolysis in Endometriosis (EMs) by our group. Under hypoxic condition, HIF-1α translocates to the nucleus, creates a dimeric complex with its partner HIF-1β subunit, and combines with the hypoxia response element (HRE), which eventually triggers the expressions of target genes. It has been confirmed that there was HRE (s) in PHB gene.
Study design, size, duration: The cultured Endometrial stromal cells were transfected with HIF-1α -siRNA. The relationship between HIF-1α and PHB were investigated by the chromatin immunoprecipitation (CHIP).
Participants/materials, setting, methods: Endometrial stromal cells (ESCs), from women with and without endometriosis, were cultured *in vitro* under normoxic and hypoxic conditions. siRNA transfection was used to down-regulate the expression of HIF-1a. Expression of PHB in those cells was quantified by Western blot. The combination between HIF-1a and PHB was explored by the chromatin immunoprecipitation (CHIP).
Main results and the role of chance: PHB level was significantly decreased in the ESCs from EMs women when compared with women without EMs (P < 0.05). In the cells from women with EMS, PHB level was decreased in the ectopic stomal cells compared with eutopic stomal cells (P < 0.05). PHB level was significantly decreased in ESCs cultured in hypoxic condition when compared with those cultured in normoxic condition (P < 0.05). Expression level of PHB was significantly increased in the ESCs when down regulated HIF-1a (P < 0.05). ChIP assays show that HIF-1α binds to PHB.
Limitations, reasons for caution: More further experiments, like luciferase reporter assays and real-time PCR, are needed to support our study. And our research is only in view of the aerobic glycolysis in EMs.
Wider implications of the findings: This finding may be a potential therapeutic target in Endometriosis for future clinical application.
Trial registration number: We do not have it.

P-312 A comparison of the changing pattern of 4 different endometrial markers of receptivity across the window of implantation in artificial cycles

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Study question: Does the 4 known markers of endometrial receptivity, pinopode, integrim αvβ3, HOXA10 and LIF change in the same pattern across the implantation window in women underwent artificial cycle?
Summary answer: The expression of all 4 markers reached a peak on the sixth days of progesterone administration.
What is known already: Interim αvβ3, HOXA10, and LIF are universal accepted implantation markers, but the clinical value of pinopode measurement in human beings remained elusive.
Study design, size, duration: This was a single-center, prospective observational study carried out in a university-affiliated reproductive center between September 2013 and October 2014. Endometrial biopsy was obtained from 39 subjects.
Participants/materials, setting, methods: Women underwent mock hormone replacement treatment cycle which had been proven capable of supporting success implantation. Endometrial samples were obtained 3 to 7 days after the initiation of progesterone therapy (P3 to P7): P3, n = 6; P4, n = 6; P5, n = 7, P6, n = 13, and P7, n = 7. The expression of pinopodes was determined by morphometric techniques, and the expression of integrin αvβ3, HOXA10 and LIF was measured with the use of semi-quantitative western blot methods.
Main results and the role of chance: (1) Although fully developed pinopodes could be observed across P3 to P7, clustered area with >50% of fully developed pinopodes could be observed from P5 to P7, the peak percentage of fully developed pinopode occurred on P6 in all cases. (2) The changing pattern of interim αvβ3, HOXA10, and LIF expression exhibited a similar trend, with a

peak on P6. (3) The expression of fully developed pinopode showed significant ($p < 0.05$) correlation with the other three markers.
Limitations, reasons for caution: None.
Wider implications of the findings: The expression of pinopodes as determined by morphometric techniques correlated well with other implantation markers. The expression of all 4 markers reached a peak 6 days after the initiation of progesterone therapy. It remains to be seen if the use of multiple markers could improve the assessment of endometrial receptivity.
Trial registration number: None.

P-313 Unique mRNA/miRNA/LncRNA signature of the endometrium during the window of implantation in patients with repeated implantation failure

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Study question: Is the mRNA/miRNA/LncRNA signature of the endometrium during the window of implantation of women with impaired endometrial receptivity different from those having normal endometrial receptivity?
Summary answer: The mRNA/miRNA/LncRNA signature of the endometrium during the window of implantation was altered in patients with RIF.
What is known already: High endometrial receptivity in the window of implantation (WOI) is essential in successful implantation. Therefore, appropriate endometrial receptivity evaluation for embryo transfer is critical for pregnancy, especially for treating patients with repeated implantation failure (RIF). However, a diagnosing tool with high specificity for impaired endometrial receptivity remains to be developed.
Study design, size, duration: Study design: A cohort of endometrial samples during the WOI was used to analysis the mRNA/miRNA/LncRNA signature of impaired and normal endometrium.
Size: 22.
Duration: 12 months.
Participants/materials, setting, methods: Participants: Patients with a history of RIF (12) and those who conceived after the first attempt of IVF/ICSI (10) were recruited as the study and control group respectively.
Setting: IVF center.
Methods: We collected endometrium specimen in WOI from all the participants. 7 and 5 samples from the study and control group respectively were conducted mRNA, miRNA and LncRNA microarray. The other samples were used for real-time PCR validation. Relevant comparative and functional analyses were performed.
Main results and the role of chance: Microarray analysis revealed 357 mRNAs, 105 miRNAs and 197 LncRNAs exhibiting modified expression between the two groups. The major function biological pathways for down-regulated mRNAs were the cytokine-cytokine receptor interaction, the p53 signaling pathway and the complement and coagulation cascades. Up-regulated mRNAs were found to mainly participate pathways such as the PPAR signaling pathway, the hematopoietic cell lineage, the phosphatidylinositol signaling system, the ECM-receptor interaction and the notch signaling pathway. A total of 176 regulations between the dysregulated miRNAs and mRNAs were found by regulatory network analysis.
Limitations, reasons for caution: The sample size is small. No less than 10 samples per group were used for the microarray analysis in our study. Considerable individual differences may exist and more samples were needed to do validation.
Wider implications of the findings: The unique profile we got can be used to develop an array, which will help to improve diagnosing the endometrial receptivity of patients with RIF.
Trial registration number: No.

P-314 Spontaneous fertility after expectant or surgical management of rectovaginal endometriosis with/without ovarian endometrioma: a 7-year retrospective analysis

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Study question: Which is the spontaneous pregnancy rate (SPR) in women with rectovaginal endometriosis (RV) with/without ovarian endometrioma (OMA) treated by expectant or surgical management?
Summary answer: Crude SPRs (cSPRs) are significantly lower in women treated by expectant versus surgical management, while adjusted SPRs (aSPRs) are similar in the two study groups.
What is known already: No evidence is available on the SPRs of women with RV with/without OMA. This lack of knowledge prevents the physician to offer these patients a proper counselling on the possibility to achieve a spontaneous conception.
Study design, size, duration: This was a retrospective analysis of a prospectively collected database (January 2009–December 2015). The study included patients with RV without OMA who either directly tried to conceive (group eRV; $n = 121$) or tried to conceive after surgery (group sRV; $n = 96$), and patients with RV with OMA who either directly tried to conceive (group eOMA; $n = 163$) or tried to conceive after surgery (group sOMA; $n = 125$).
Participants/materials, setting, methods: The study included patients with RV with/without OMA, without history of infertility and with partners with normal semen analysis. Patients tried to conceive spontaneously for one year either without undergoing surgery or after surgery. cSPRs (calculated according to intention-to-treat analysis), aSPRs (calculated considering only patients who tried to conceive consecutively for one year), time to conception, and pregnancy outcomes were compared between the study groups.
Main results and the role of chance: At one year, cSPRs was lower in group eRV (24.8%) than in group sRV (42.7%; $p = 0.004$). Similarly, cSPRs was inferior in group eOMA (11.7%) than in group sOMA (30.4%; $p < 0.001$). 57.9% (group 1; 70/121 patients), 22.9% (group 2; 22/96 patients), 70.6% (group 3; 115/163 patients), and 24.8% (group 4; 21/125 patients) gave up trying to conceive spontaneously before 1-year follow-up. At one year, no significant difference was observed in aSPRs between group eRV (58.8%) and group sRV (55.4%; $p = 0.423$), and between group eOMA (39.6%) and group sOMA (40.4%; $p = 0.535$). Furthermore, at 1-year follow-up, cSPRs was significantly higher in group eRV (24.8%) than in group eOMA (11.7%; $p = 0.003$), and in group sRV (42.7%) than in group sOMA (30.4%; $p = 0.040$). At the same follow-up, aSPRs was significantly higher in group eRV (58.8%) than in group eOMA (39.6%; $p = 0.043$), and in group sRV (55.4%) than in group sOMA (40.4%; $p = 0.038$).
Limitations, reasons for caution: This research is limited by the retrospective study design. Furthermore, the SPRs reported in this study were observed in selected populations of women without history of infertility and with partners with normal semen analysis. Therefore, these data cannot be extrapolated to the general population of women with endometriosis.
Wider implications of the findings: If further studies will confirm the results of this research, patients with RV with/without OMA might be advised that surgery improves cSPRs. The presence of OMA decreases cSPRs independently from expectant or surgical management.
Trial registration number: Not applicable.

P-315 Morpho-functional endometrium peculiarities after uterine artery embolisation in leiomyoma patients with reproductive plans

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Study question: Are there morphological and functional changes in endometrium in leiomyoma patients after uterine artery embolisation (UAE) without myoma node expultion reducing fertility?
Summary answer: UAE more often leads to chronic endometritis, hypoplasia and dyshronosis of endometrium, decrease in expression of nuclear estrogen receptor and Ki-67 in glands and stroma.
What is known already: UAE for leiomyoma is becoming accepted as an alternative to surgical treatment but still to be controversial issue for women with incomplete reproductive plans. Ovarian failure and uterine infection are the most

dreaded complications of this procedure. The frequency of abnormal hysteroscopic findings after embolization is surprisingly high. In experiment UAE was associated with a decrease in endometrial integrin αvβ3 levels, which would negatively affect endometrial receptivity, but might be transient and reversible. Conflicting results varying from completely well tolerated to negative effect on endometrium and ovary function have been reported. Nevertheless, pregnancies after this procedure have been described.

Study design, size, duration: Prospective study of 125 patients of Reproductive Health Institution during 2012–2015. Among them 91 leiomyoma patients 20 to 45 years old admitted for organ-safe surgery: 48 patients with multi-nodes leiomyoma of big size with advanced blood flow where EUA was performed (Gr1); 43 patients with laparotomy conservative myomectomy (Gr2). Exclusion criteria: suspicion of oncology, PID or node expulsion after UAE, entry into the uterus during myomectomy. 34 IVF patients without leiomyoma served as control (CGr).

Participants/materials, setting, methods: Hysteroscopy with biopsy of uterus and cervix was performed for all patients (as part of IVF protocol). In 6 months after surgery "second look" biopsy was performed. Morphology; immunohystochemestry of endometrium: CD-138, nuclear estrogen (ER) and progesterone (PR) receptors in glands and stroma, Ki-67; scan electronic microscopy (SEM) were studied. Descriptive statistics was used.

Main results and the role of chance: Before surgery: endometrium structure corresponds to the phase of the menstrual cycle – 9 (18.75%)- Gr 1; 8 (18.6%)- Gr 2; 29 (85.29%)- CGr (pGrI-CGr < 0.05: pGr2-CGr < 0.05); polyps – 11 (22.91%); 11 (25.58%) and 1 (2.94%) respectively (pGrI-CGr < 0.05: pGr2-CGr < 0.05); simple hyperplasia- 4 (29.16%) in Gr1 and 12 (27.9%) in Gr2 (pGr1-Gr2 > 0.05); complex hyperplasia in 4 (8.33%) and 4 (9.3%), respectively, no cases in CGr.

6 months after surgery: endometrium polyps – 5 (10.41%) in Gr1 and 1 (2.35%) in Gr2, ($p > 0.05$); dischronosis of endometrium in 9 (18.75%) and 1 (2.35%) patients respectively ($p < 0.05$).

After UAE there was endometrium hypoplasia in 3 (6, 25%) patients, morphological signs of chronic endometritis in 12 (25%), positive expression of CD 138 in stroma in 15 (31, 25%). No cases of hypoplasia and chronic endometrities after conservative myomectomy.

There was increase CD16 and CD56 in stroma expression and decrease in RE and Ki-67 expression in glands and stroma in Gr1 ($p < 0.05$) and decrease in expression of nuclear steroid receptors. CD 138, CD 16, CD 56, Ki-67 in gland and stroma in Gr2 comparing to CGr ($p > 0.05$).

Rare pinopodii reduced dimensions on SEM, implantation window displaced more than 3 days were found in Gr1.

Limitations, reasons for caution: Not the same indications were considered for UAE and conservative myomectomy. UAE is admitted to women with reproductive intentions only in case it is the only possible way to save uterus.

No difference in endometrium status before the surgery in two groups (Gr1 and Gr2) make comparison after surgery possible.

Wider implications of the findings: UAE can be considered as the only option to preserve uterus in women with reproductive plans in big size multiple fibroids with advanced blood flow. High frequency of dangerous endometrium pathology after UAE requires hysteroscopy in 6 months. Future investigation is needed for in-time diagnosis and correction of endometrium receptivity.

Trial registration number: No trial; this prospective study was approved by Ethics Committee.

P-316 Magnetic resonance enema versus computed tomographic colonography in the diagnosis of bowel endometriosis

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Study question: Which is the performance of magnetic resonance enema (MRe) and computed tomographic colonography (CTC) in the diagnosis of bowel endometriosis?

Summary answer: Both MRe and CTC have high accuracy in the diagnosis of intestinal endometriosis without significant difference between the two techniques.

What is known already: Several radiological techniques have been proposed for the diagnosis of intestinal endometriosis. Transvaginal ultrasonography is now considered the first line investigation in the study of rectosigmoid endometriosis; however, it cannot assess the presence of intestinal nodules located proximally to the sigmoid because these lesions are beyond the field of transvaginal ultrasonography. It remains to be established which is the best radiological technique to study the large intestine.

Study design, size, duration: This double-centre prospective study included 105 women with suspicion of intestinal endometriosis who underwent laparoscopy. Patients were examined and underwent surgery between October 2013 and January 2016.

Participants/materials, setting, methods: This study included women of reproductive age scheduled for laparoscopy with suspicion of intestinal endometriosis. Patients were excluded from the study if they had previous surgical diagnosis of intestinal endometriosis, previous radiological diagnosis of intestinal endometriosis, history of colorectal surgery (except appendectomy), previous bilateral ovariectomy and contraindications to bowel preparation or CTC. CTC and MRe results were compared with surgical and pathologic findings. Different radiologists independently and blindly performed CTC and MRe.

Main results and the role of chance: The mean (±SD) age of the study population was 34.9 (±5.5) years. At the time of the study, 69 patients (65.7%) were under hormonal therapy. 59 patients (56.2%) had surgical diagnosis of intestinal endometriosis. The accuracy, sensitivity, specificity, positive likelihood ratio, negative likelihood ratio, positive predictive value and negative predictive value for CTC and MRe were, respectively, 90.1%, 89.8% (79.2%–96.2%), 92.2% (81.1%–97.8%), 11.5 (4.5–29.5), 0.1 (0.1–0.2), 93.0% (83.0%–98.1%), 88.7% (77.0%–95.7%) and 92.8%, 91.5% (81.3%–97.2%), 96.1% (86.5%–99.5%), 23.3 (6.0–91), 0.1 (0.0–0.2), 96.4% (87.7%–99.6%), 90.7% (79.7%–96.9%). The McNemar's test showed that there was no significant difference in the diagnostic performance of the two techniques in the diagnosis of intestinal endometriosis ($p = 606$). 9 patients had multicentric intestinal endometriosis (multiple lesions affecting different digestive segments); MR-e identified multicentric disease in 8 patients and CTC in 7 patients ($p = 0.782$). 3 patients had cecal lesions, which were identified by CTC in all the cases and by MR-e only in one patient ($p = 0.619$). No adverse events occurred during CTC and MR-e; no patient required to interrupt the exams. The intensity of pain experienced during CTC was higher than the intensity of pain perceived during MR-e ($p < 0.001$).

Limitations, reasons for caution: Despite the large sample size of the study, few patients had multicentric disease and cecal lesions; future studies should assess the accuracy of the two techniques in diagnosing these intestinal nodules. The administration of radiation (although in low-dose) is a disadvantage of CTC.

Wider implications of the findings: Depending on the experience of the radiologist, both MR-e and CTC can be used to diagnose intestinal endometriosis. MR-e should be preferred to CTC because the patients better tolerate the exam and it does not required to expose the patients to radiation.

Trial registration number: Not applicable.

P-317 Pregnancy outcomes in women with endometriosis with and without adenomyosis: a 4-year retrospective study

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Study question: Does adenomyosis influence the outcomes of pregnancy in women with endometriosis?

Summary answer: In women with endometriosis, diffuse but not focal adenomyosis increases the risk of small for gestational age babies (SGA), intrauterine growth restriction (IUGR), preterm birth.

What is known already: Several studies investigated the correlation between endometriosis and pregnancy outcome complications such as placenta previa, preterm birth, SGA, IUGR, gestational diabetes, pregnancy-induced

hypertension (PIH) and preeclampsia. However, no previous study compared these pregnancy outcomes in patients with endometriosis with and without adenomyosis.

Study design, size, duration: This is a retrospective analysis of a prospectively collected database during a four-year period. The study included 457 patients with endometriosis, among these patients 323 did not have adenomyosis, 82 had focal adenomyosis and 52 had diffuse adenomyosis.

Participants/materials, setting, methods: This study included patients with any form of endometriosis; the diagnosis was performed either by surgery and histology or by transvaginal ultrasonography. Adenomyosis was diagnosed by ultrasonography, it was classified in focal or diffuse. The study included patients who conceived spontaneously or by assisted reproductive techniques.

Main results and the role of chance: The three groups were similar in demographic characteristics (age, body mass index, parity, race, mode of conception). Endometriosis was histologically confirmed in 312 (68.3%) patients. Patients with diffuse adenomyosis and endometriosis had an increased risk of SGA (OR = 5.14, 95% CI 1.71–15.47; $p = 0.004$), IUGR (OR = 5.68, 95% CI 2.34–13.76; $p < 0.001$) and preterm birth (OR = 2.21 CI 95% 1.04–4.69; $p = 0.039$) compared with those with endometriosis without adenomyosis. Patients with diffuse adenomyosis compared with those with endometriosis only had similar other pregnancy outcomes: placenta previa (OR = 1.04, 95% CI 0.30–3.65; $p = 0.954$), gestational diabetes (OR = 0.52 95% CI 0.18–1.50; $p = 0.223$), PIH (OR = 1.14 CI 95% 0.24–5.27; $p = 0.872$), preeclampsia (OR = 2.11 CI 95% 0.42–10.76; $p = 0.368$). Patients with focal adenomyosis had similar pregnancy outcomes compared with those with endometriosis only: SGA (OR = 0.98, 95% CI 0.21–4.73; $p = 0.984$), IUGR (OR = 1.55, 95% CI 0.54–4.48; $p = 0.419$), placenta previa (OR = 1.10, 95% CI 0.40–3.06; $p = 0.855$), preterm birth (OR = 1.14 CI 95% 0.54–2.42; $p = 0.727$), gestational diabetes (OR = 0.96 95% CI 0.47–1.95; $p = 0.904$), PIH (OR = 0.35 CI 95% 0.45–2.75; $p = 0.318$), preeclampsia (OR = 0.65 CI 95% 0.77–5.49; $p = 0.694$).

Limitations, reasons for caution: The sample size is the main limitation of this study. Another potential limitation of the study is that the diagnosis of adenomyosis (in all the patients) and of endometriosis (in a proportion of the patients) was made by ultrasonography without histological confirmation.

Wider implications of the findings: The findings of this study suggest that pregnancies of patients with endometriosis and diffuse adenomyosis are more likely to be associated with SGA and IUGR; therefore, these pregnancies may need closer monitoring.

Trial registration number: Not applicable.

P-318 Effect of human endometriotic cyst fluids on mouse *in vitro* folliculogenesis and oocyte acquisition

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Study question: Does the supplementation of human endometriotic cyst fluids (EMF) have a detrimental effect on *in vitro* follicle growth and oocyte acquisition in a mouse model.

Summary answer: Supplementation of human EMF negatively affects *in vitro* follicle growth, hormonal production and oocyte acquisition.

What is known already: An ovarian endometrioma contains free iron, reactive oxygen species, proteolytic enzymes and inflammatory molecules in concentrations from tens to hundreds of times higher than those present in peripheral blood or other types of benign cysts. The endometrioma is not surrounded by a true capsule, thus contents of endometrioma appear to across the lining cyst wall. It was postulated that the presence of an endometrioma per se may cause direct damage to the surrounding otherwise healthy ovarian tissues.

Study design, size, duration: Prospective experimental animal study. Supernatants of human EMF were added to the growth and maturation medium containing preantral follicles obtained from 7- to 8-week-old BDF1 mice. A total of 409 preantral follicles were randomly divided into four groups according to the final concentrations of human EMF; 0%, 2.5%, 5%, and 10%.

Participants/materials, setting, methods: After *in vitro* growth and maturation of mouse preantral follicles for 12 days, survival rate of follicles, oocyte acquisition, meiotic spindle integrity of MII oocytes and hormonal levels of 17β-estradiol and anti-Müllerian hormone (AMH) in the final spent media were assessed.

Main results and the role of chance: The survival rates of follicles at day 10 were significantly lower in three EMF-treated groups (56.1%, 30.6%, and 6.2%, respectively, $p < 0.05$ for each when compared with 83.6% in 0% EMF group). The acquired total oocytes (34.7%, 18.4% and 4.1%) or MII oocytes (10.2%, 3.1%, and 1%) per initiated follicle were also significantly lower in three EMF-treated groups ($p < 0.05$ for each when compared with 68.1% and 21.6% in 0% EMF group). Meiotic spindle was severely damaged in almost all of MII oocytes acquired from three EMF-treated groups. The level of 17β-estradiol was significantly lower in the 10% EMF-treated group and the levels of AMH were significantly lower in three EMF-treated groups when compared with those in 0% EMF group.

Limitations, reasons for caution: We used a mouse model to demonstrate a detrimental effect of human EMF on *in vitro* folliculogenesis.

Wider implications of the findings: Our results suggest that contents of ovarian endometrioma could affect directly folliculogenesis in the adjacent ovarian tissues. Our results can greatly contribute to understand the mechanism of endometrioma-induced ovarian damage.

Trial registration number: This work was supported by grant no. A120043 from the Korea Health Care Technology R&D Project, Ministry of Health and Welfare, Republic of Korea.

P-319 Freeze-all policy improves clinical outcome in patients with recurrent implantation failure: A randomized controlled trial

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Study question: Does a freeze-all policy of *in-vitro* human blastocysts improve the clinical outcome in patients with recurrent implantation failure (RIF)?

Summary answer: There was a statistically significant improvement in the clinical outcome in freeze-all group over the fresh embryo transfer (ET) group, in cases of RIF.

What is known already: There is a growing evidence that elective frozen-thawed embryo transfer in a non-stimulated cycle (freeze-all policy) would eliminate the risk of COS and resulting in better endometrial receptivity and lower uterine contractility as compared with fresh ICSI cycles. Many management approaches attempted to enhance ICSI outcome in cases of recurrent implantation failure, which aimed to improve quality of the embryos, receptivity of the endometrium and the embryo-endometrium interaction. However, there are no reports studied the effect of freeze all policy in improving the clinical outcome in patients with RIF.

Study design, size, duration: This randomized controlled study was conducted from February 2012 to August 2015 on 200 women with RIF, at least three failed previous ICSI attempts with at least 8 good embryos transferred under the age of 40 years. Patients were randomized to either the fresh ET group (control arm) or the freeze all group (study arm) according to a computer-generated list. Informed consent was obtained before allocation from all patients.

Participants/materials, setting, methods: Women were randomly divided in two groups of 100 women each. All patients underwent the same controlled ovarian hyperstimulation long protocol. Couples with testicular or epididymal sperm were excluded. Only Day 5 cycles resulted in blastocysts were included in this study. Cryopreservation of human blastocysts for freeze all group has been employed using vitrification, and then transferred in a consecutive natural cycle. Pretransfer morphology of blastocysts was assessed according to Gardner and Schoolcraft grading system.

Main results and the role of chance: No statistically significant differences were found in demographic data between the fresh ET group and freeze all group, including female age (33.52 ± 3.72 and 32.92 ± 3.34, respectively, $P =$

0.231), female BMI (24.85 ± 0.85 and 25.03 ± 1.38, respectively, $P = 0.267$), duration of infertility (6.72 ± 2.35 and 7.2 ± 2.11, respectively, $P = 0.13$), and number of previous ICSI attempts (3.8 ± 0.99 and 4.0 ± 0.94, respectively, $P = 0.144$). There was no statistically significant difference in endometrial thickness between patients of both groups (Fresh ET group 10.4 mm; Freeze all group 10.1 mm, $P = 0.351$).

The mean number of blastocysts transferred was similar between the two groups (Fresh ET group 2.28 ± 0.73; Freeze all group 2.08 ± 0.8). Quality of transferred blastocysts showed no significant differences between the fresh ET group and freeze all group [(Grade A blastocysts: 47.33% and 42.67, respectively, $P = 0.35$), (Grade B blastocysts: 30% and 37%, respectively, $P = 0.112$), and (Grade C blastocysts: 24.67% and 18.33%, respectively, $P = 0.196$)]. Clinical pregnancy rate (Fresh ET group 28%; Freeze all group 52%) and Ongoing pregnancy rate (Fresh ET group 20%; Freeze all group 44%) were statistically significant ($P = 0.001$). The implantation rate was also significant (Fresh ET group 17.0% and Freeze all group 44.33%; $P = 0.001$).

Limitations, reasons for caution: Further large scale multicentre randomised controlled studies are required to confirm our findings.

Wider implications of the findings: The results showed, for the first time, improvement in the clinical pregnancy, implantation, and ongoing pregnancy rates freeze-all policy group over the fresh ET group, in cases of RIF. Thus, newly developed freeze all policy may now offer an alternative option to improve the implantation efficiency for patients with RIF.

Trial registration number: NCT02681367.

P-320 Follicular phase endometrial stimulation (FES) in the transfer cycle in RIF cases: a Randomized control trial

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Study question: Does follicular phase endometrial stimulation in the transfer cycle improve the clinical pregnancy in comparison to that performed in the luteal phase in recurrent implantation failure (RIF) cases?

Summary answer: Follicular phase endometrial stimulation (FES) in the transfer cycle is beneficial in RIFs showing a higher trend in the clinical pregnancy and implantation rates.

What is known already: Scrapping of endometrium (Endometrial stimulation/biopsy) in the previous non-transfer cycle is one of the strategies to improve the implantation and clinical pregnancy rate in RIF cases. It leads to release of growth factors, cytokines, induces endometrial decidualization, increases angiogenesis all of which could lead to an improved outcome. The objective of this RCT was to test the hypothesis if endometrial stimulation performed in the follicular phase of transfer cycle could improve the probability of pregnancy in patients who have had two previous failed IVFs.

Study design, size, duration: This prospective randomized, double blinded, proof of concept study was conducted in a tertiary care center from August 2013 to December 2015 in 304 RIF cases defined as 2 previous failed ETs (fresh or frozen) with transfer of at least four good-quality embryos (grade 1) in women less <37 years. Block randomization was done with sealed envelope system using random number table. At recruitment written informed consent was taken. Approval taken from Institutional Ethical Board.

Participants/materials, setting, methods: Endometrial stimulation (ES) was performed using a Pipelle biopsy (Gynetics, Belgium) on outpatient basis. Group A: (Intervention group) – ES performed on day 4–6 of transfer cycle ($n = 49$ fresh cycles; 51 = frozen cycles); Group B: (Intervention group)- ES performed on day 20–22 of previous cycle ($n = 49$ fresh cycles; 51 = frozen cycles. Group C: (Control group)- no ES in previous 3 cycle ($n = 49$ fresh cycles; 51 = frozen cycles).

Main results and the role of chance: [I] Fresh cycles: Of the 151 patients recruited, 4-refused to participate. The baseline, stimulation and embryological characteristics were similar in all 3 groups. 147 patients underwent stimulation of which 22 patients [Group A ($n = 6$); Group B ($n = 9$); Group C ($n = 7$)] did not undergo a fresh embryo transfer and hence were excluded for the analysis.

[II] Frozen embryo transfer (FET) cycles: Of the 153 patients, cycles were canceled in 13 patients [Group A ($n = 4$); Group B ($n = 5$); Group C ($n = 4$)] and hence excluded. The age, BMI and endometrial thickness, the mean number of embryos thawed, survived and transferred were similar in all 3 groups. The sub-endometrial blood flow was better in group A reaching up to zone 3–4 [GA (2.98 ± 0.87); GB (2.67 ± 0.89); GC (2.26 ± 0.82); $p = 0.09$].

[III] Outcomes: (a) Primary outcome: Although the results did not show a statistical significance in the fresh cycles, a trend towards a better clinical pregnancy was seen in group A ($p = 0.09$). In the FET cycles, the clinical pregnancy was statistically significant ($p = 0.04$) in group A.

(b) Secondary outcome: The implantation rate in the fresh cycles was [GA: 23/90 = 25.5%; GB: 15/92 = 16.3%; GC: 13/92 = 14.1%; ($p = 0.07$)] showing a trend towards better implantation in group A. In the FET cycles, this was statistically significant ($p = 0.04$).

Limitations, reasons for caution: Limitations of the study was the sample size and the live birth rate. Live birth rate is being collected and will be reported when available.

Wider implications of the findings: The study demonstrates a better cycle outcome in the intervention group (A and B) compared to no intervention group (C) in RIFs with FES in the transfer cycle showing a higher trend towards clinical pregnancy and implantation. However large, multicenter randomized studies are needed to confirm the above findings.

Trial registration number: None.

P-321 Comparison of intracytoplasmic sperm injection (ICSI) cycles outcome in two groups of patients with endometriosis with and without sclerotherapy

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Study question: Among aspiration ethanol sclerotherapy (AEST) and previouse laparoscopic surgery on treating endometrioma which modality is the preferred option with a more favorable outcome in intracytoplasmic sperm injection (ICSI) cycles.

Summary answer: Although sclerotherapy for endometrioma does not negatively affect implantation and pregnancy rates in embryo transfer cycles, recurrence of endometriosis is higher compared to laparoscopic surgery.

What is known already: The performance of laparoscopic surgery and AEST for treating endometrioma have been separately reported in earlier studies. However, the outcome of ICSI cycles for number and quality of oocytes, implantation rates, pregnancy rates and recurrence of endometrioma have not been concurrently compared in the two method.

Study design, size, duration: 61 patients which enrolled with infertility and endometrioma undergoing ICSI cycles from March 2014 to till January 2016.

Participants/materials, setting, methods: Women with infertility and endometrioma divided in two groups. In group 1, 31 patients received ICSI cycles and simultaneous sclerotherapy. In group 2, 30 patients with endometrioma were treated by laparoscopic surgery. If no pregnancy occurred after one year, for all the patients controlled ovarian stimulation and ICSI was used. In group1, ultra-sound guided aspiration of endometrioma and AEST was performed then ICSI was done. Number and quality of oocytes, implantation rates, pregnancy rates and recurrence of endometrioma were compared between the two groups.

Main results and the role of chance: The number of retrieved oocytes were 216 in group 1 (mean 6.96) and 146 (mean 4.8) in group 2. The number of mature oocytes were 184 in group 1 (85%) and 124 (84%) in group 2. The implantation rate for group 1 was 5.03% and.6.4% for group 2. The pregnancy rates were 22% and.20% for group 1 and group 2 respectively. Implantation rate in group 1 was 4.5% in fresh embryo transferred cycles versus 5.2% in freeze embryo transferring. No abortions occurred in any of the patients. One-year follow-ups revealed that none of the patients in group 2 showed evidence of recurrent disease but in one-third of patients in group 1, recurrence of endometrioma was detected.

Limitations, reasons for caution: Limitation of the study is number of cases, which if number of cases are increased, the results may be different.

Wider implications of the findings: If at the beginning of the management of endometrioma, AMH (anti mullerian hormun) level is above 2 ng/ml, laparoscopic cystectomy for endometrioma is still the management of choice and should be considered for patients with endometriosis with acceptable ovarian reserve tests.
Trial registration number: None.

P-322 Randomized controlled trial to evaluate the usefulness of GnRH agonist versus placebo on the outcome of IVF in infertile patients with endometriosis

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Study question: Could improve clinical pregnancy rate a 3-month pre-treatment with GnRH agonist administered immediately before *in vitro* fertilization in infertile patients with endometriosis?
Summary answer: Regarding our study results, a 3-month pre-treatment with GnRH agonist prior IVF did not improve clinical pregnancy rate in infertile patients with endometriosis.
What is known already: Endometriosis causes infertility due to several mechanisms, such as fallopian tube obliteration and decreasing ovarian reserve. Furthermore, an inflammatory state has been demonstrated in follicular and peritoneal environment, which could impair oocyte and embryo quality and implantation rate.

Assisted reproductive techniques outcomes in endometriosis seem worse than in patients with other causes of infertility. The influence of adjuvant treatments on these outcomes has been investigated. A pretreatment with GnRH agonists before IVF could improve reproductive outcomes in terms of implantation and pregnancy rates in patients with endometriosis. However, most of the studies performed to date are not prospective and randomized.
Study design, size, duration: Prospective controlled, double-blind, randomized clinical trial.

The duration was of 36 months (between March 2012 and March 2015). Two hundred patients were enrolled.

Clinical and embryological outcomes were analyzed, as well as stimulation parameters. Clinical pregnancy rate was the primary endpoint.
Participants/materials, setting, methods: Patients: Two hundred infertile women with endometriosis diagnosed by laparoscopy and/or the presence of endometrioma on vaginal ultrasound were randomized into two groups.

Setting: Human Reproduction Unit. La Fe University Hospital.

Interventions: 3.75 mg triptorelin (group 1) or placebo (group 2) was administered on days 1, 28 and 56 after menstrual cycle. Ovarian stimulation, oocyte retrieval, fertilization, embryo culture and embryo transfer were performed according standard protocols, and were identical in both groups.
Main results and the role of chance: One hundred and eighty three patients were included for statistic analysis (Group 1, $n = 92$ and group 2, $n = 91$). Seventeen patients were excluded: two because of adverse effects, six withdrawn voluntarily, two because of screening failure, and seven because of protocol violation.

The mean duration of infertility was 2.9 years (±1.5). The average patient age was 33.7 years (±3.2, range 22–39). The mean BMI was 22.6 kg/m^2 (±2.8). The mean basal FSH level was 7.1 IU/L (±1.9).

Analyses of the covariates showed that duration of infertility, age, BMI, basal FSH and stage of endometriosis were not significatively different in both groups.

The amount of gonadotropins needed to ovarian stimulation and the number of stimulation days were significantly higher in group 1: 3002 IU (±949) vs. 2346 IU (±657), and 10.4 days (±2.6) vs. 9 days (±1.7), respectively in groups 1 and 2 ($p = 0.000$).

Estradiol levels on the day of administration of hCG were 1896.9 pg/mL (±765.8) and 2218.2 pg/mL (±795.4), respectively in groups 1 and 2 ($p = 0.000$).

Number of total oocytes, metaphase II, fertilized oocytes and embryos did not show statistical significative differences.

The clinical pregnancy rate was 25% and 34.1%, respectively in groups 1 and 2 ($p = 0.179$).

Limitations, reasons for caution: Patients affected of all stages of endometriosis have been included. However, partial analysis of data did not show statistical differences neither in the distribution of patients in both groups nor in IVF outcomes.

In 37 cases, surgical procedures took place more than 3 years prior to inclusion in the study.
Wider implications of the findings: According to these results, there is no indication for pre-treatment with GnRH agonist in infertile patients with endometriosis in order to improve IVF outcomes. This treatment increases both ovarian stimulation duration and total amount of gonadotrophins requiered, whithout improving pregnancy rate.
Trial registration number: Clinicaltrials.gov: NCT01581359 ID: ENDOFIV-010.

P-323 Clinical outcome after endometrial scratching (ES) in IVF-patients with a history of implantation failure

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Study question: Does ES prior to embryo transfer improve clinical outcome in IVF-patients with a history of implantation failure?
Summary answer: ES prior to blastocyst transfer might contribute to successful implantation in IVF-patients with a previously failed IVF attempts.
What is known already: Successful nidation of an embryo depends, beside embryo quality on the receptivity of the endometrium. The endometrium is a highly complex and dynamic tissue that undergoes several morphological and molecular changes during the menstrual cycle. Several studies indicate that ES might improve endometrial receptivity. Possible explanations include the secretion of different cytokines and growth factors, promotion of neo angiogenesis and at least alternations of endometrial gene expression which might improve synchrony between endometrium and blastocyst. However, the application of ES is not without controversy. Criticisms include amongst others the heterogeneity of studies and small numbers of patients included.
Study design, size, duration: We conducted a retrospective analysis of cycles with ES prior to embryo transfer (ET) in the time period between January 2010 and June 2015. Inclusion criteria for ES were at least one failed IVF-attempt in a previous cycle and blastocyst transfer. Clinical outcome of 190 ET was evaluated. Results after ES were retrospectively compared to patients previous in-house cycles before ES. Final outcome parameters were pregnancy rate (PR), birth rate (BR) and miscarriage rate (MR).
Participants/materials, setting, methods: Mean female age was 36.8 years (27–43). Mean number of failed IVF-cycles/patient prior ES was 4.1 ± 2.4, mean total number of ET/patients prior ES was 4.6 ± 2.5. The PR/ET rate in previous IVF-cycles prior ES was 12.7% (112/883). Generally, ES was performed once in the mid-luteal phase of the cycle prior ET. Progesterone was injected IM for luteal phase support. Blastocyst transfer was done on day 5 using a Wallace transfer catheter.
Main results and the role of chance: Following ES, a mean number of 1.8 blastocysts were transferred on day 5. We observed a PR of 29.5% (56/190). In 24.7% of patients clinical pregnancy was confirmed by positive heart beat (47/190). In 7 patients a miscarriage was reported (7/47). BR was 21.1% (40/190) including 33 singletons and 7 twins. In a sub-analysis only patients with ≥3 failed IVF-cycles were included (126 ET). In this sub-group a PR of 26.0% was obtained compared to 9.6% in previous cycles without ES. After ES in 23.0% of the patients PR was confirmed by positive heart beat and a BR of 15.9% was observed. No adverse effects of ES were observed. Our results indicate that patients with recurrent implantation failure (RIF) might benefit from ES.
Limitations, reasons for caution: This study is one of the largest performed on this topic. However, this is a retrospective study and non-randomized with its inherent limitations. Our results have to be confirmed by a randomized controlled trial before ES should be proposed as beneficial for patients with a history of implantation failure.
Wider implications of the findings: To the present day it is not unequivocally clear whether ES is helpful for ART patients and which sub-population of infertility patients might profit from this procedure. This large retrospective study is further indication that ES might support embryo implantation in patients with RIF.
Trial registration number: Not applicable.

P-324 Effect of prolonged gonadotropin releasing hormone (GnRH) agonist before IVF versus placebo on CYP19 A1 gene expression in granulosa cells in infertile women with endometriosis

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Study question: The expression of CYP19A1 gene was compared in mural granulosa (MGC) and cumulus cells (CC) in infertile women with endometriosis pre-treated with GnRH agonist prior IVF.
Summary answer: No significant difference in the expression of CYP19A1 gene in MGC nor CC was observed between groups after administration of GnRH agonist before IVF.
What is known already: Aromatase plays a fundamental role in the establishment of oocyte quality, which might be compromised in endometriosis. Some authors reported decreased aromatase activity in granulosa cells in women with endometriosis, which might lead to defects in steroideogenesis and abnormal oocyte functioning. Data on the expression of the aromatase gene in luteinized MGC are controversial and only one study evaluated the expression in CC and found reduced expression in CC of infertile women with endometriosis.

On the other hand, it has been suggested that the administration of GnRH agonist for a few month prior to IVF increases the pregnancy rate.
Study design, size, duration: A prospective blinded randomized trial was performed at our tertiary care assisted reproductive program. Sample included 40 infertile women with endometriosis. All patients were candidates for IVF-ET. Patients were randomized consecutively from October 2013 to March 2015 in two groups by using a computer-generated randomization: 20 women received GnRH agonist and 20 placebo every 28 days for three injections.
Participants/materials, setting, methods: Patients included had an indication for ART due to endometriosis diagnosed by laparoscopy and/or the presence of endometrioma on vaginal ultrasound. Samples were obtained individually only from the two aspirated follicle of each ovary. CYP19A1 gene expression in MGC and CC was quantitated through real-time polymerase chain reaction and follicular fluid concentration for estradiol, testosterone and androstenedione were measured. Oocyte and embryo quality were determined through morphological parameters and fertilization rate was calculated.
Main results and the role of chance: Both groups did not differ significantly regarding baseline clinical data in terms of age, serum FSH and estradiol level. Patients with minimal/mild endometriosis represented 72%.

Total gonadotropin dose were significantly higher in study group compared with control group (FSH (UI) = 2923.53 ± 862.11 and 2325.00 ± 764.39) ($p = 0.04$) and total number of fertilized oocytes (3.22 ± 1.99 and 5.21 ± 3.60) ($p = 0.04$) and fertilization rate (58.92 ± 28.25 vs. 77.23 ± 20.44) ($p = 0.03$) were significantly lower in the study group, respectively.

No significant differences were found between groups in the expression of the CYP19A1 in MGC (ΔΔMGC = 1.44 ± 1.85 and 1.23 ± 3.19) and CC (ΔΔCC = 2.15 ± 2.99 and 2.61 ± 3.96), respectively. The groups did not differ in CYP19A1 expression when compared MGC to CC.

Follicular hormones leves were lower in study group compared with control group (E2 (pg/ml) = 293495.4 ± 154806.6 vs. 400520.284892.9, Testosterone (ng/ml) = 3.10 ± 2.44 vs. 9.38 ± 7.94 and Androstenedione (ng/ml) = 6.86 ± 4.66 vs. 10.90 ± 11.67, respectively), although only testosterone level was significantly lower ($p = 0.00$).

The groups did not differ with regard to oocyte and embryo morphological parameters, although a trend toward lower embryo quality was found in the study group.
Limitations, reasons for caution: This randomized trial focused only in the evaluation of oocyte and embryo quality in patients with endometriosis after administration of GnRH agonist for 3 month before controlled ovarian hyperstimulation.

However, GnRH agonist appear to have other beneficial effects that may increase fecundity in patient with endometriosis through other mechanisms.
Wider implications of the findings: The admnistration of GnRH agonist did not confirm the potential hypothesized mechanism by which CYP19A1 aberrant expression influences the adquisiton of oocyte competence. However, we found lower follicular hormonal levels that might lead to abnormal oocyte functioning and favour the low number of fertilized oocytes and fertilization rate found.
Trial registration number: Clinicaltrials.gov: NCT01581359 ID: ENDOFIV -010.

P-325 Galectin-3 inhibitors as targeted therapy for endometriosis

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Study question: Can pathways involved in Endometriosis be targeted for non-invasive therapy for the disease?
Summary answer: Galectin-3 inhibitor is a potential novel targeted future therapeutic in endometriosis. These compounds are already in clinical use as potent inhibitor of blood malignancies treatments.
What is known already: Galectin-3 is overexpressed in Endometriosis. Galectin-3 antagonists are potent inhibitor of blood malignancies, reducing mcl-1 and bcl-x_L whilst inducing noxa, upregulates p21, downregulates cyclin D1, and interrupts NFκB and akt signalling. It has no myelotoxicity and an acceptable toxicity profile, increasing the evidence of the potential use of a galectin-3 inhibitor as a novel therapeutic medication for women in their reproductive age, with Endometriosis.
Study design, size, duration: Initial TMAs on 200 endometriosis samples, in a prospective study, confirmed upregulation of Galectins and CyclinD1 levels. *In vitro* therapeutic experiments were carried out on primary ovarian and peritoneal endometriosis cells as well as normal uterine endometrial cells (96 well plate cultures) to assess for cellular response to Galectin-3 inhibitors in endometriosis and normal cells at varying concentrations of drug.
Participants/materials, setting, methods: Primary ovarian, peritoneal endometriosis and normal endometrial cellular samples were cultured in 96 well plates from a pooled cohort of otherwise healthy women (30.75 years) undergoing laparoscopic examination. Cultured cells were treated with the galectin-3 inhibitor at specified concentrations *in vitro*. Cellular viability response was observed using a fluorescence well plate reader. Fluorescence outputs for each well were assessed for cellular viability and compared between treated and untreated cells at various concentrations and across timelines.
Main results and the role of chance: Increased levels of Galectin-3 and Cyclin D1 in endometriosis tissues were confirmed with Tissue Microarrays on a set of 200 histologically confirmed endometriosis samples.

Ovarian endometriosis, peritoneal endometriosis and normal endometrial uterine cells were placed separately into 96 well plates for therapeutic studies. The drug at a specified concentration was added to each well containing viable cells in culture. Each therapeutic experiment was run in triplicate to strengthen result validity. At 72 h post treatment with Galectin-3 inhibitor there was an observed direct dose dependant relationship between treated endometriosis cells and percentage cell survival. The binding of Galectin-3 has shown a role in anti-apoptosis, anti-angiogenesis and inhibited cellular migration. Untreated cells maintained viability supporting this Galectin inhibitor as a potential targeted future non-invasive therapeutic in endometriosis.
Limitations, reasons for caution: Further *in vivo* modelling is required. The Galectin-3 inhibitor tested is however already in use clinically in CLL patients for the treatment of malignancy. Its safety profile in humans in known and the profile suggests it could be safe for use in young women of reproductive age.
Wider implications of the findings: Unlike current suppressive medical treatments, or invasive surgical techniques, the use of a therapeutic that directly inhibits endometriosis proliferation will potentially provide a cure for this progressively debilitating disease in young women.
Trial registration number: Ethical approved research and study (Ethics reference REC 10/H0711/24).

P-326 The menstrual phase independent proteome of the endometrium of women with endometriosis is distinctly different from women without – results from quantitative proteomic analysis

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Study question: 1) Is the proteome of the endometrium of women with endometriosis different from those without endometriosis? 2) Are the differences menstrual phase independent?
Summary answer: Proteomic profiling of the endometrium from women with and without endometriosis has uncovered novel distinctly differentially up and down regulated proteins as potential candidate biomarkers.
What is known already: A number of studies have analysed the proteome of the endometrium of women with endometriosis ($n = 15$). However, due to methodological variation, studies have not managed to achieve consensus on their results. The inconsistencies were due to utilisation of techniques that 1) have high variability and 2) identify proteins at a low dynamic range.
Study design, size, duration: Sixteen endometrial samples [endometriosis (EN), $n = 8$; control (CN), $n = 8$] were collected between September 2013 and September 2015. There was no significant difference ($P > 0.05$) in age ($P = 0.829$, EN, 33.1 ± 4.35; CN, 32.5 ± 6.63), body mass index ($P = 0.88$, EN, 25.4 ± 4.60; CN, 25.7 ± 4.17) and baseline FSH [$P = 0.51$, EN, 6.9 ± 1.90; CN, 7.5 ± 1.83) between the two groups.
Participants/materials, setting, methods: Endometrial tissue was homogenised, proteins extracted and then reduced, alkylated and trypsin proteolysed. Peptides were iTRAQ labeled and analysed using 2D LC-MS proteomics.
Main results and the role of chance: Nearly 10,000 proteins were profiled from each of the two datasets in this study. Between the two analyses, 210 and 235 proteins were commonly up and down regulated respectively. The highly up regulated proteins were Liver carboxylesterase-1 (CES1) was significantly up regulated ($Log_2Ratio = 0.5$), Homeobox protein-B6 (HOXB6, $Log_2Ratio = 0.5$), Synaptotagmin-like protein 1 (SYTL-1, $Log_2Ratio = 0.4$), Tumour associated signal transducer 2 (TACSTD2, $Log_2Ratio = 0.5$), Microsomal Glutathione S-transferase 2 (MGST2, $Log_2Ratio = 0.5$) and Cluster Differentiation 99 (CD99, $Log_2Ratio = 0.5$). Adiponectin (ADIPOQ, $Log_2Ratio = –0.6$) was significantly down regulated across both datasets. Bioinformatics interpretation showed that phagocytosis of cells ($p = 0.0013$; activation z-score = –2.2) and migration of macrophages was predicted to be inhibited ($p = 0.01$; activation z-score = –2.0) in the endometrium from patients with endometriosis compared to controls.
Limitations, reasons for caution: The number of sample used for this study is small but sufficient for a biomarker discovery phase. Although the sample were thoroughly washed and cleaned during tissue procurement, there is always a possibility of contamination with blood that will interfere with the protein analysis.
Wider implications of the findings: Without the exclusion of low abundance proteins, our study has uncovered novel non-hormonally related proteins in the endometrium of women with endometriosis. Further verification and validation will be required using larger sample size using ELISA or immunohistochemistry.
Trial registration number: Nil.

P-327 Follicular fluid (FF) of infertile women with endometriosis causes poor oocyte maturation by inducing higher reactive oxygen species (ROS) and DNA damage in mouse oocytes

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Study question: What are the factors affecting poor oocyte yield in women with endometriosis? Can FF cause poor oocyte maturation rate in endometriosis women and how does FF cause oocyte maturation arrest?
Summary answer: IFF from women with endometriosis causes increased metaphase I arrest but is reversible by inhibitors of oxidative stress, the DDR pathway or the SAC.
What is known already: FF contains factors that contribute to a healthy environment for oocyte maturation process within a developing follicle. Abnormal FF can affect the maturation process and cause poor oocyte yield in women with endometriosis when undergoing IVF/ICSI. This can lead to poor reproductive outcomes. In bovine oocyte, FF of women with endometriosis causes maturation arrest but the mechanism underlying the arrest is unknown. Higher Reactive oxygen species (ROS) in endometriosis may cause DNA damage and affect oocyte maturation process.
Study design, size, duration: FF from follicles containing mature oocytes were obtained from 16 infertile women between January and December 2014 during oocyte retrieval. Immature mouse oocytes were incubated without FF (No-FF), with control FF (Ct-FF) from patients with no endometriosis, or with FF from patients with mild (ME-FF) and severe (SE-FF) endometriosis.
Participants/materials, setting, methods: *In Vitro* maturation was performed using FF and maturation media in 1:1 ratio with and without time-lapse imaging. Oocytes were fixed and immunostained for visualisation of chromosomes, spindles, DNA damage and PBE after 14–16 h.
Main results and the role of chance: 1,668 immature mouse oocytes were incubated for 14–16 h in media with 15% or 50% FF. At 15%, 92/167 (55.1%) oocytes extruded a polar body in SE-FF group and was significantly lower compared to No-FF (132/190, 69.5%, $P = 0.001$, 95%CI) and Ct-FF (88/132, 66.7%, $P = 0.0442$, 95%CI). More marked effect demonstrated at 50% SE-FF (150/309, 48.5%) when compared to media with No-FF or with Ct-FF (No-FF, 216/286, 75.5%, $P < 0.0001$; Ct-FF, 172/242, 68.4%, $P = 0.0001$, 95% CI, Fisher's exact test]. There was delayed onset of PBE timing ($P > 0.05$) and arrested oocytes were mainly ($P = 0.008$) at Metaphase I without any evidence of abnormal spindle morphology or incorrect spindle attachment ($P > 0.05$). There was an increase intracellular ROS uptake in SE-FF group compared to No-FF ($P < 0.0001$, MD 0.81 [0.41 to 1.21] 95%CI; Ct-FF, $P = 0 < 0.0001$, MD –1.01 [–1.38 to –0.64] 95%CI) and higher level of DNA damage one-hour incubation with FF from women with endometriosis compared to (No-FF $P < 0.0001$, MD 0.9 [0.49 to 1.32] 95%CI; MF-FF, $P = 0.023$, MD –0.45 [–0.88 to –0.03] 95%CI) after exposure to SE-FF. Maturation rate reverted to normal ($P > 0.05$ vs. Control) when inhibitors to Spindle Assembly checkpoint (SAC), DNA damage response (DDR) or ROS were added in the media containing SE-FF.
Limitations, reasons for caution: This study is using animal models and may not necessarily be extrapolated to humans.
Wider implications of the findings: This study has shown that the decrease number of oocyte retrieved in women with endometriosis is related to the metaphase I arrest but a reverseable process. This has important clinical implications on *in vivo* and *in vitro* therapeutic interventions that may potentially improve the reproductive outcome of those with endometriosis.
Trial registration number: REC13/SC/0213.

P-328 Serum 17-alpha-hydroxyprogesterone (Sr. 17-α-OHP) level on day of blastocyst transfer: a discerning indicator of endometrial receptivity and successful implantation in IVF cycles?

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Study question: To evaluate serum 17-α-OHP level on day of blastocyst transfer and explore its feasibility as a compelling marker of endometrial receptivity compared to endometrial thickness in IVF cycles.
Summary answer: Serum level of 17-alpha-hydroxyprogesterone (17-α-OHP) on day of blastocyst transfer is a pertinent indicator of enhanced endometrial response favorable for implantation in IVF cycles.
What is known already: Inadequate uterine receptivity is responsible for approximately two-thirds of implantation failures. Sonography, hysteroscopy, endometrial biopsy are considered the anatomical and functional markers of endometrial receptivity. An earlier study had reported significance of transvaginal ultra-sonographic evaluation of endometrial thickness on pregnancy rates. However, since the ovarian steroid dependent process of human embryo implantation occurs during a stringent window period; analysis of molecular mediators involved at the embryo-uterine junction during this phase may

give better insight into the functional assessment of endometrial receptivity. Although, endometrial biopsy samples are being evaluated, nevertheless, an in-cycle online marker of receptivity and implantation is still elusive.
Study design, size, duration: Prospective clinical study without randomization of 111 IVF cycles in women (mean age 30.86 ± 3.98 years) undergoing standard ovarian stimulation protocol with antagonist. Study was carried out from April 2014 to March 2015 at our IVF centre. All cycles involved day 5 blastocyst transfer. Serum 17-α-OHP levels were measured by radioimmunoassay on day of blastocyst transfer (dBT). Main outcome measure was Embryo Implantation Rate (EIR = total number of embryo sacs observed/total number of blastocycts transferred x 100).
Participants/materials, setting, methods: Luteal phase was supported with micronized Progesterone injection. βhCG >50 mIU/ml on day 14 post transfer was considered as positive indicator of implantation. Gestational sac with positive cardiac activity confirmed clinical pregnancy. Cycles were divided into Implantation success vs. Implantation failure depending on whether conception occurred or not and into Low and High dBT 17-α-OHP groups depending on median value of dBT 17-α-OHP levels. Statistical analysis was done using Graph-pad prism V software.
Main results and the role of chance: Overall clinical pregnancy rate was 28.83% (32/111) and embryo implantation rate was 20.32% (38 implantations/total 187 blastocysts transferred). Implantation/Conception success cycles ($n = 32$) showed significantly higher levels of 17-α-OHP on dBT compared to No-Implantation/Non-conception cycles ($n = 79$) {10 ng/ml vs. 7 ng/ml, $\chi 2$ $p = 0.01$}. Embryo implantation rates were significantly higher in the high (≥7.88 ng/ml, $n = 53$) dBT 17-α-OHP group compared to low (<7.88 ng/ml, $n = 58$) dBT 17-α-OHP group {31% vs. 11%, $\chi 2$ $p = 0.0058$}. 17-α-OHP levels on dBT correlated significantly with implantation (Spearman $r = 0.32$). Implantation failure occurred in 97.92% of cases in low group compared to 49.06% in the High dBT 17-α-OHP group giving an odds ratio and relative risk of no implantation as 4.44 and 1.65 ($p = 0.0004$) respectively. Conversely, the odds ratio and relative risk in favour of implantation in the High group was 4.44 and 2.69 respectively ($p = 0.0004$). Incidentally, endometrial thickness did not differ significantly between the low and high dBT 17-α-OHP groups (1.11 vs. 1.08 cm, $p = 0.46$) so also in the implantation success vs. implantation failure cycles (1.11 vs. 1.09 cm, $p = 0.64$). Understandably therefore, dBT endometrial thickness correlated very poorly with implantation (Spearman $r = 0.076$, $p = 0.44$). 17-alpha-Hydroxyprogesterone is thus a more convincing indicator of implantation and endometrial receptivity in ongoing IVF cycles.
Limitations, reasons for caution: Larger multicentric studies are required to establish 17-alpha-hydroxyprogesterone as a robust biomarker of implantation and endometrial receptivity.
Wider implications of the findings: Progesterone secreted by corpus-luteum gets converted to 17-α-OHP which in turn may lead to formation of estrogens required for development of secretary endometrium rendering it receptive for implantation. Measurement of dBT 17-α-OHP level may facilitate our clinical decision on whether to transfer embryo in same cycle or subsequent favourable cycle.
Trial registration number: Not Applicable.

P-329 Additional benefit of fibrin sealant patch in preservation of ovarian reserve during laparoscopic ovarian cystectomy

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Study question: Is additional hemostasis by hemostatic fibrin sealant patch (FSP) superior to that achieved by bipolar coagulation (BC) only in preserving ovarian reserve in patients undergoing laparoscopic ovarian cystectomy?
Summary answer: In women with bilateral endometriomas, post-operative anti-Müllerian hormone (AMH) levels, was less diminished when ovarian hemostasis was obtained combining FSP and BC versus BC only.
What is known already: There is a consensus that surgical excision of ovarian endometriomas may damage ovarian reserve. The methods used to obtain the hemostasis after stripping of endometriomas might influence ovarian reserve.
Study design, size, duration: This study was based on a retrospective analysis of a prospectively collected database of patients who underwent laparoscopic stripping of unilateral (UE; $n = 30$) or bilateral endometriomas (BE; $n = 20$). After surgical excision of endometriomas, hemostasis was obtained either by BC or by minimal BC plus the application of FSP (Tachosil, Takeda, Rome, Italy) according to surgeons' preference.
Participants/materials, setting, methods: This study included women undergoing laparoscopic stripping of unilateral or bilateral endometriomas with largest diameter ≥4 cm. Exclusion criteria were: age ≥40 years, previous surgery on the ovaries or for endometriosis, previous oophorectomy. Ovarian reserve was assessed before surgery and at 6 months from surgery by measuring antral follicle count (AFC) and serum AMH. The prevalence of ovarian adhesions was assessed by ultrasonography at 6 months from surgery.
Main results and the role of chance: The mean age of the study population was 32.5 (±3.6) years. Both in patients with UE and in those with BE, the baseline AFC ($p = 0.773$ and $p = 0.764$, respectively) and the AMH levels ($p = 0.941$ and $p = 0.824$, respectively) were similar the two treatment groups. In patients with UE, the AFC of the operated ovary did not change after surgery both in patients treated by BC ($p = 0.419$) and in those treated by FSP ($p = 0.659$); at 3-month follow-up, the AFC of the operated ovary was similar between the two treatment groups ($p = 0.814$) and also AMH levels did not differ (0.548). In patients with BE, the total AFC did not change after surgery both in patients treated by BC ($p = 0.398$) and in those treated by FSP ($p = 0.840$), while a significant reduction in AMH levels was observed in both in patients treated by BC ($p = 0.002$) and in those treated by FSP ($p < 0.001$); at 3-month follow-up, the total AFC was similar between the two treatment groups ($p = 0.444$), while AMH levels were significantly higher in patients treated by FSP versus BE (0.031). In addition, the use of FSP did not increase the prevalence of postoperative adhesions both in patients with UE ($p = 0.337$) than in those with BE ($p = 0.110$).
Limitations, reasons for caution: The main limitation of the current study is the retrospective design. Another important limitation of this research is the small sample size.
Wider implications of the findings: In women undergoing surgical excision of bilateral endometriomas, the use of FSP provides a potential additional benefit in the preservation of ovarian reserve. Future studies in larger population of patients should confirm these preliminary results.
Trial registration number: Not applicable.

P-330 The relation between spontaneous hemoperitoneum in pregnancy (SHiP) and endometriosis – data from a nationwide Dutch consorted action

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Study question: What can we learn from the largest case series of SHiP (Spontaneous Hemoperitoneum in Pregnancy), especially when SHiP is related to endometriosis?

Summary answer: Clinicians should be aware of SHiP when pregnant women with a history of endometriosis present with severe abdominal pain, hypovolemic shock or fetal distress.

What is known already: Spontaneous Hemoperitoneum in Pregnancy is a rare, but potentially life-threatening complication, which occurs predominantly during the third trimester of pregnancy. SHiP is associated with adverse pregnancy outcome for both mother and child. The etiology is largely unknown, but endometriosis is the major risk factor for SHiP. Since pregnancies in endometriosis patients are more often facilitated by assisted reproductive techniques, the incidence of SHiP may increase. Therefore, a greater awareness of adverse pregnancy outcomes in these women is essential.

Study design, size, duration: In collaboration with the members of the Dutch Working Group on Endometriosis we identified unpublished unique cases of SHiP that occurred in the Netherlands in the period 2008–2015. The data are retrieved from the original patient files. For this case series, no approval of the institutional review board was required. Patients' written consent to publish was obtained in all cases.

Participants/materials, setting, methods: The risk of excessive bleeding from pelvic endometriosis during pregnancy is presented by 15 events of SHiP in 11 women diagnosed with endometriosis. Recurrence of SHiP, present in four of these cases, is a novel finding in the literature.

Main results and the role of chance: SHiP occurred predominantly in the second ($n = 4$) and third trimester of pregnancy ($n = 5$), but also in the first trimester ($n = 1$), during labor ($n = 3$) or several weeks postpartum ($n = 2$). In four cases recurrence of SHiP was described, in one of these cases the second event of SHiP occurred in a consecutive pregnancy. The earliest and major presenting symptom was an acute onset of abdominal pain, often combined with a fall in hemoglobin level. Ultrasound examination or MRI was helpful in observing free peritoneal fluid in ten cases. When recurrences of SHiP are counted separately, midline laparotomy was performed in nine episodes of SHiP, of which four were converted from a laparoscopy. Pfannenstiel laparotomy was performed in four cases. Two cases did not require immediate intervention and expectant management was chosen. The necessity of surgical interventions ($n = 13$) in pregnancy ($n = 11$) were due to maternal indications ($n = 7$), fetal indications ($n = 2$) or both ($n = 2$). No maternal or perinatal mortality was reported, despite the urgency of a cesarean section at preterm age (27–34 weeks gestation, $n = 5$). In ten cases endometriosis was causal in the pathogenesis of SHiP. However, in the remaining case endometriosis was confirmed several years later.

Limitations, reasons for caution: Since the exact prevalence of SHiP is unclear, a nationwide registration network is warranted in order to record cases of SHiP in a prospective way. This network (NethOSS) is currently set up and will allow us to give an exact incidence number for the Dutch population.

Wider implications of the findings: This case-series shows that awareness and adequate multidisciplinary intervention results in absent maternal and perinatal mortality, although a high percentage of preterm births remains. In order to further improve the outcome, growing knowledge of this serious complication is advocated. Appropriate counseling should be considered when these women want to conceive.

Trial registration number: Not applicable.

P-331 Impaired endometrial receptivity is the possible cause of infertility in women with genital tuberculosis – retrospective analysis of 689 patients with tubal infertility undergoing IVF

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Study question: What is the leading cause of infertility in women with genital tuberculosis (GTB)- tubes or the endometrium?

Summary answer: Endometrium is probably the main cause of infertility in GTB.

What is known already: GTB invariably affects the fallopian tubes and endometrial involvement is noted only in about 50% of the cases. It results in infertility by affecting the tubes (tubal obstruction and dysfunction), reducing implantation, forming uterine synechia and least commonly due to ovulatory failure. Successful pregnancy is very low even after complete treatment in GTB. *In-vitro* fertilization (IVF) remains the most successful treatment modality in these women. As GTB impairs implantation by reducing endometrial receptivity markers, there is a low pregnancy rate even after IVF.

Study design, size, duration: Retrospective analysis of case records of infertile women with tubal factor infertility registered for IVF at our tertiary hospital and reproductive health research unit between November 2011 and January 2015. 689 patients between the ages of 25 and 40 with tubal factor infertility (excluding endometriosis) undergoing first IVF cycle were analyzed. Tubercular tubal infertility (GTB group) and non tubercular tubal factor infertility (NTB group) was found in 277 patients 412 patients respectively.

Participants/materials, setting, methods: 246 patients in GTB and 398 in NTB group who completed their IVF cycle were evaluated. Endometrium of 88 patients who failed to conceive following IVF cycle but had either miscarriage or recurrent implantation failure in the past was collected and analyzed. Endometrial receptivity including αvβ3 integrin, E-cadherin, MECA-79, mucin-1, pinopodes and leukemia inhibitory factor (LIF)/LIF receptor- signal transducers and activators of transcription 3 (STAT3) expressed in the endometrium were analyzed and compared with fertile group.

Main results and the role of chance: Analyzing past obstetric history, we noted a higher miscarriage rate in women with GTB (50/246) while occurrence of ectopic pregnancy (23/398) was more common in NTB group. In 31 patients of GTB group embryo transfer was cancelled, of which 16 cycles were due to thin endometrium (<6 mm). Cycle characteristics were comparable in both groups in terms of demographic profile, dose of gonadotropin, number of oocytes retrieved, number of embryos formed but endometrial thickness was significantly lower in GTB than NTB group (9.5 ± 2.09 vs. 10.08 ± 1.5; $p < 0.0001$). Doppler indices like end diastolic velocity ($p < 0.05$) was significantly less and systiolic/diastolic ratio ($p < 0.0001$) significantly higher in GTB group. Pregnancy rate was significantly lower in GTB group than NTB (17.07 vs. 35.67; $p < 0.0001$). Live birth rate was significantly lower (5.28% vs. 24.62%; $p < 0.0001$) but biochemical pregnancy (7.72% vs. 2.26%; $p < 0.0013$) was significantly higher in GTB group in comparison to NTB group. Significantly reduced levels of endometrial receptivity markers LIF/LIF receptor, and STAT3 signaling pathway were observed in endometrium of women who underwent endometrial sampling in comparison to fertile control. *In vitro* studies confirmed our findings demonstrating that 65 kDa mycobacterial heat shock protein (HSP65) reduced decidualization of human endometrial stromal cells (hESC) significantly via decreased LIF-STAT3 signaling.

Limitations, reasons for caution: This is a retrospective study and we didn't compare the endometrial receptivity markers between NTB and fertile group. Furthermore, various other cytokines and related receptor-mediated signaling pathways were not evaluated in the present study.

Wider implications of the findings: Past miscarriage history, increased chances of biochemical pregnancy and low live birth rate after IVF in GTB group suggests possible role of endometrial affliction in GTB. Further prospective trials are needed to fully understand the cause of implantation failure in patients with genital tuberculosis patients.

Trial registration number: Not applicable.

P-332 Meta-analysis of endometrial receptivity-associated genes

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Study question: To identify the meta-signature and putative biomarkers of receptive endometrium.

Summary answer: Using a novel meta-analysis approach, we identified a meta-signature of receptive endometrium involving 57 genes.

What is known already: Previous microarray-based transcriptome studies of the endometrium have revealed hundreds of simultaneously up- and down-regulated genes that are involved in endometrial receptivity. However, the overlap between the studies is relatively small, and we are still searching for

potential diagnostic biomarkers. Numerous limitations of microarray technology together with differences in study design have made it difficult to perform a meta-analysis that would identify potential biomarkers with sufficient power and reliability/credibility.
Study design, size, duration: We performed a systematic review and meta-analysis of transcriptome studies on pre-receptive- and receptive-phase endometria from healthy fertile women.
Participants/materials, setting, methods: A systematic review of the literature was conducted up to October 2015 in PubMed and Scopus databases. Of the final 14 eligible studies, nine were included in the meta-analysis. The pooled dataset obtained represented 164 endometrial samples – 76 from pre-receptive- and 88 from receptive-phase endometria. The up- and down-regulated genes were ranked by their fold changes. A robust rank aggregation (RRA) algorithm was used for meta-analysis of the gene lists, followed by enrichment analysis.
Main results and the role of chance: We identified a meta-signature of endometrial receptivity involving 57 genes as putative receptivity markers, including *ANXA4*, *APOD*, *CD55*, *CLDN4*, *COMP*, *DPP4*, *EDN3*, *GADD45A*, *GPX3*, *HABP2*, *IGFBP1*, *IL15*, *MAOA*, *MMP7*, *PAEP*, *SLC1A1*, *SPP1* and *S100P*. The meta-signature genes highlight the importance of immune responses, the complement cascade pathway and the involvement of extracellular vesicles in mid-secretory endometrial function.
Limitations, reasons for caution: Because of the limited number of gene lists in the meta-analysis, the RRA results are likely to be influenced by sampling bias and depend heavily on each dataset used in the analysis.
Wider implications of the findings: The identified meta-signature genes and pathways could serve as promising and sought-after biomarkers of endometrial receptivity and pregnancy establishment, and also markers of uterine pathologies.
Trial registration number: 0.

P-333 Epigenetic analysis of aromatase coding gene, CYP19A1, in endometrial tissue of women with endometriosis during menstrual cycle

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Study question: Is there any correlation between altered expression of *CYP19A1* gene with epigenetic changes on its promoter region, in endometrium and endometriotic lesions of women with endometriosis?
Summary answer: Hyper-acetylation and hypo-methylation of *CYP19A1* regulatory regions are in correlation with increased level of aromatase gene expression, which may contribute to the progression of endometriosis.
What is known already: Epigenetic aberrations such as DNA methylation and histone modifications appear to be involved in various diseases such as endometriosis. Endometriosis is defined as the presence of endometrium like tissue outside of uterine. It is proofed that endometriosis is an estrogen dependent disease and one of the key enzymes in estrogen biosynthesis is aromatase. Aromatase encoded by the gene *CYP19A1,* has 10 tissue specific promoters on its regulatory region. Here, we investigated epigenetic alteration of **PII and PI.3** promoters of *CYP19A1* and its correlation with mRNA expression during menstrual cycle.
Study design, size, duration: This is a case-control laboratory study in which, ectopic lesions and eutopic endometrium samples were collected by laparoscopy from 20 women with documented endometriosis. As a control group, endometrial tissues were collected from 20 healthy fertile women who underwent laparoscopy for tubal ligation surgery during menstrual cycle. Informed consent was collected from each participant.
Participants/materials, setting, methods: Relative expression analysis of *CYP19A1* was evaluated by quantitative real-time PCR. The DNA binding of MeCP2 and specific histone modifications in **PII and PI.3** promoters region of *CYP19A1* gene were examined by chromatin immunoprecipitation (ChIP) technique.
Main results and the role of chance: Expression profile of *CYP19A1* gene revealed significant increase ($p < 0.05$) in mRNA level in ectopic lesion and eutopic endometrium in patients *vs.* control group, during menstrual cycle. In endometriosis patients, incorporation of MeCP2 in promoter **PII** of *CYP19A1* was significantly lower than control group ($p < 0.05$), which showed a harmonious pattern between mRNA expression of *CYP19A1* and methylation level of its promoter region (PII). Significant hyperacetylation of lysine 9 of histone 3 (H3K9ac) of promoters **PII** and **PI.3** observed in patients affected endometriosis ($p < 0.05$). Furthermore, significant methylation level at lysine 9 of histone 3 (H3K9me2) of promoter **PI.3** was detected between patients and control group ($p < 0.05$), whereas no significant difference of methylation was detected in **PII** promoter.
Limitations, reasons for caution: For getting more information we need to investigate large number of endometriosis patients and control group, and also evaluate epigenetic alterations of *CYP19A1* in other promoters of this gene.
Wider implications of the findings: Our results showed that high expression of *CYP19A1* in patients with endometriosis might be due to epigenetic alterations through regulatory region of *CYP19A1,* either through DNA methylation or histone modification. Also, results showed probable epigenetic switch between PII and pI.3 promoters of *CYP19A1* gene in endometriosis patients.
Trial registration number: NA.

P-334 Collagen for sustained release of growth factors for treating patients with poorly developed thin endometrium

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Study question: Does collagen enable sustained release of growth factors and improve their treatment effect on poorly developed thin endometrium of infertile women?
Summary answer: Collagen enables sustained release of epidermal growth factor (EGF) and improves the treatment effect of EGF on poorly developed thin endometrium of infertile women.
What is known already: Although several treatment modalities have been offered to patients with thin endometrium, treatment for them is still challenging. Several growth factors and cytokines are required for proper preparation of the endometrium. Granulocyte colony-stimulating factor (G-CSF) and EGF have been shown to cure patients with thin endometrium. However, study on sustained release of growth factors for treating patients with thin endometrium has not been reported.
Study design, size, duration: *In vitro* release test of EGF and granulocyte-macrophage colony-stimulating factor (GM-CSF) was performed. In addition, we analyzed the results of a total of 66 consecutive infertile women with thin endometrium <6 mm despite estradiol treatment who underwent intrauterine treatment with either EGF-loaded collagen solution (study group, $n = 37$) or EGF alone solution (control group, $n = 29$) between March 2014 and November 2015 for this retrospective study.
Participants/materials, setting, methods: For *in vitro* release test, 0.8% collagen gel was mixed with EGF or GM-CSF solution in phosphate buffered saline (PBS). The growth factor-loaded gels were incubated in PBS at 37 °C and supernatant was taken at 4, 8, 24, 48 and 72 h after mixture. In the study group, slow intrauterine infusion of 0.8% collagen in which 50 ng/ml EGF was loaded was performed every 2–3 days during the follicular phase of 3–4 menstrual cycles.
Main results and the role of chance: Percentages of released EGF and GM-CSF from 0.8% collagen gel at 24 h after mixture were 58.5 ± 3.7 and 51.9 ± 2.3%. Percentages of released growth factors at 72 h was significantly higher in EGF of 83.5 4.8% compared with 58.3 ± 3.0% in GM-CSF loading ($p < 0.001$). Endometrial thickness after EGF treatment compared with before treatment significantly increased in both study and control groups ($p < 0.001$, $p < 0.01$, respectively). When study and control groups were compared after treatment, endometrial thickness was significantly higher in the study group ($p = 0.002$) and resistance index (RI) of subendometrial artery (SEA) was significantly lower in the study group ($p < 0.001$).
Limitations, reasons for caution: Compared with EGF, released amount of GM-CSF from collagen gel was much smaller. In addition, our study may have a limitation to evaluate the effect of EGF-loaded collagen solution on thin endometrium due to a small number of sample available and retrospective nature.
Wider implications of the findings: This is the first study to evaluate the effect of collagen on sustained release of growth hormones for intrauterine treatment

of thin endometrium. This new treatment regimen may have a new therapeutic potential for infertile women with poorly developed thin endometrium.
Trial registration number: No.

P-335 Establishment of a new 3D model to investigate the embryo-maternal interface

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Study question: Can a 3D model of the embryo-maternal interface be established that resembles the *in vivo* situation better than the commonly applied 2D models?
Summary answer: We were able to establish a viable 3D two spheroid model of maternal and embryonic cells.
What is known already: Common human cell culture models of early implantation processes are mostly based upon 2D cell models of maternal cells which are incubated with embryonic spheroids or embryo secretory products so far. Further possibilities contain artificial media/groundings – for example matrigel – allowing a 3D proliferation and growth.
Study design, size, duration: Control (same cell type either maternal or embryonic) versus treatment (maternal and embryonic spheroids); 8 performances in each condition; treatment duration 0–120 h.
Participants/materials, setting, methods: 2D culture of maternal endometrial stromal cells (cell line St-T1; kind gift of Prof. Jan Brosens[1]), endometrial cells with knock-down of Syndecan-1 (KdS1[2]) and embryonic cells (HTR-8/SVneo; ATCC®CRL-3271™) for proliferation. For spheroid formation, 3×10^4 St-T1 cells and 2×10^4 HTR-8 cells were cultured in hanging droplet culture for 48 h. In order to identify maternal and embryonic cells, these were incubated either with MitoTracker® or stained with vimentin or cytokeratin 7. Immunohistochemistry and spheroid confocal laser microscopy.
Main results and the role of chance: The spheroid formation worked well and robust and was repeatable severalfold. In order to measure implantation objectively, we performed central point distance measurements between the spheroids. After 12 h of confrontation of maternal stromal and embryonic cells the distance was reduced significantly. The knock-down of Syndecan-1 resulted in a shortened, but still statistically significant converge of the different cell types.
Limitations, reasons for caution: Only *in vitro*.
Wider implications of the findings: This new model enables insight into the early embryo maternal interaction due to its unique resemblance of the *in vivo* situation compared to the established 2D models. In upcoming investigations, signaling pathways will be evaluated to reveal the cellular impact on the interaction.
Trial registration number: nn.

P-336 GnRHa induces both apoptosis and autophagy of endometriotic tissues by down-regulation of estradiol levels in women with endometriosis

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Study question: To determine whether endometrial cell autophagy is altered by aberrant mTOR activity and is associated with apoptosis in endometriotic cysts treatment with GnRH agonist (GnRHa).
Summary answer: GnRHa simultaneously induces apoptosis by upregulating Bax/Bcl-2 Ratio and autophagy by enhancing the conversion of LC3-I to LC3-II in human endometriotic tissue.
What is known already: GnRH analogs (GnRHa) are the most widely used hormonal treatments for endometriosis. Nevertheless, the effect of GnRHa treatment on the eutopic endometrium of patients with endometriosis and the activation of apoptosis through these events remain unknown. In this study, we illustrated the effects of GnRHa in patients with endometriosis after GnRH agonist therapy to identify proteins that might provide further information concerning the mechanisms underlying the functions of GnRHa.
Study design, size, duration: A prospective controlled study in patients with stage III-IV endometriosis in a university hospital. The study group was treated with GnRHa at least 1 month before laparoscopic surgery ($n = 15$), and the control group ($n = 15$) without GnRHa treatment. Tissue specimens were collected from both groups during laparoscopic surgery. In flow cytometric analysis, we used CRL-7566 endometriosis cell line (ATCC, Manassas, VA, USA). Cells were pre-treated with estradiol (E2) before the treatment with or without GnRHa.
Participants/materials, setting, methods: The expression levels of apoptosis- (Bax/Bcl-2 ratio, p-mTOR, p-Akt and cleaved caspase 3) and autophagy-related proteins (LC3-II and Beclin-1) with or without GnRHa therapy were determined by Western blot, IHC, and flow cytometric analysis using Annexin V-FITC and PI staining. The data were expressed as the mean ± SD of 3 or more independent experiments and statistical analysis was performed using Student's *t*-test.
Main results and the role of chance: GnRHa treatment induced apoptosis via upregulation of Bax/Bcl-2 ratio ($p < 0.05$), Annexin V binding capacity and caspase-3 activation in endometriosis. In addition, cytochrome c release and caspase-3 cleavage were also demonstrated by IHC staining. GnRHa induced autophagy in endometriotic tissue was shown by a significant increase in LC3-II expression, a hallmark of autophagy. Besides, GnRHa downregulated the phosphorylation of major components of mTOR pathway, such as p-Akt at Ser473, p-mTOR and its downstream substrates p-p70S6K (p70 ribosomal protein S6 kinase) and p-S6K ($p < 0.05$). E2 is a potent inhibitor of apoptosis and it regulates the expression of several apoptotic proteins, including Bcl-2 and mTOR in endometriotic tissues. Indeed, compared with GnRHa treatment, decreased mTOR activity in endometriotic tissues *without GnRHa treatment* inhibited autophagy and apoptosis induction. The present study strongly illustates that downregulation of Bcl-2, leading to an increase in the Bax/Bcl-2 ratio and subsequent activation of caspases 3 and aberrant Akt-mTOR activity in endometriotic tissues following GnRHa treatment, may be a plausible explaination which leads to simultaneously apoptosis and autophagy of endometrial cells. Therefore, it can be postulated that altered induction of autophagy by aberrant mTOR activity is a feasible mechanism that facilitates the decreased apoptosis found in endometriotic tissues.
Limitations, reasons for caution: In this study we compared the results obtained by IHC with those obtained by Western blot analysis for profiling cellular signaling pathway. However, due to the endometriotic-derived stromal cells of patients treated with GnRHa consists of very slow growing cells, it has been difficult to analyze by flow cytometry.
Wider implications of the findings: In the present study, we first investigated the therapeutic effect of GnRHa induces the simultaneous activation of apoptosis and autophagy by modulating the Akt-mTOR signaling pathway in endometriotic tissues.
Trial registration number: TMU-JIRB 201305035.

P-337 Cytokine expression profile of *in vitro* cultured endometrial stromal and epithelial cells during the window of implantation (WOI) – a promising tool for receptivity assessment

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Study question: Which cytokines are differently secreted by primary endometrial stromal and epithelial cells, cultured with and without E2 and P4, and in what quantities?
Summary answer: Significant changes in expression of IL-21, TNF-alpha, G-CSF, GM-CSF, RANTES, IL-6, HGF and IL-17A of endometrial stromal and endometrial epithelial cells were observed.
What is known already: Endometrial epithelial cells (EEC) and endometrial stromal cells (ESC) actively secrete a variety of cytokines and growth factors which is thought to be crucial for implantation in different species, including human. It is well known that EEC secrete granulocyte-macrophage

colony-stimulating factor (GM-CSF), leukemia inhibitory factor (LIF), colony stimulating factor (CSF-I) and TNF-α. ESC secrete interleukin-6 (IL-6), macrophage chemo-attractant protein-1 (MCP-1). Some growth factors and cytokines are maximally produced by EEC during the mid-secretory phase of the endometrial cycle, such as LIF, IL-11, IL-6, CSF-I, GM-CSF, CCL11, CCL14, CCL21, Glycodelin A and HB-EGF.

Study design, size, duration: In the present study, we examined the profile of 21 cytokines in (1) the conditioned media (*RPMI*-1640 supplemented with *10% FBS*) and (2) conditioned media with addition of E2 (0.1 nM) and P4 (100 mM) of primary cell cultures from human endometrial stromal cells (ESC) and epithelial cells (EEC). Endometrial cells were obtained from mid-secretory phase samples from 28 healthy women. The levels of cytokines in the culture supernatants were assayed after 48 h incubation.

Participants/materials, setting, methods: Nine cytokines (IL-4, IL-6, IL-10, IL-15, IL-17A, IL-22, TNF-alpha, IFN-gamma and GM-CSF) in the supernatants were analyzed using ELISA. Twelve cytokines [MIP-1 beta, MCP-1, VEGFA, PDGF-BB, MIG, RANTES, HGF, MMP9, EGF, G-CSF, Calcitonin and IL-12 (p70)] were analyzed by a FACS Calibur flow cytometer using FlowCytomix Multiplex Kit. Student's paired *t*-test was used to compare the changes in cytokine profile of endometrial cells cultured with and without E2 and P4.

Main results and the role of chance: Under basal conditions in the control group, endometrial stromal and epithelial cells exhibited specific profiles of secreted cytokines. Our results showed that the stromal cells secrete higher quantities of GM-CSF (ESC vs. EEC: 0.19 vs. 0.14 pg/ml), HGF (ESC vs. EEC (mean values): 2488 vs. 116 pg/ml) and significantly lower quantities of IL-6 (ESC vs. EEC: 0.18 vs. 0.28 pg/ml), IL-21 (ESC vs. EEC: 0.50 vs. 1.08 pg/ml), TNF-alpha (ESC vs. EEC: 0.19 vs. 0.39 pg/ml) and G-CSF (ESC vs. EEC: 2.75 vs. 12.38 pg/ml) in the culture medium, compared with epithelial cells. Administration of E2 and P4 resulted in significantly ($P < 0.05$) different secretion of RANTES (0.3-fold increase) and EGF (5.8-fold increase) in endometrial epithelial cells and IL-6 (1.5-fold increase), HGF (2.9-fold decrease) and IL-17A (1.3-fold decrease) in endometrial stromal cells.

Limitations, reasons for caution: A limitation of this study is the relatively low number of patients. Although the data were obtained in human primary endometrial cells, they result from *in vitro* studies. Thus, caution is needed in their translation for *in vivo* conditions. Similar analysis should be performed in patients with repeated implantation failure.

Wider implications of the findings: This study supports the importance of E2 and P4, specifically during the implantation window. Understanding the differences in levels and change in cytokine profile of *in-vitro* cultured endometrial cells could help to create a diagnostic tool for receptivity assessment and to develop treatment strategies for patients with repeated implantation failure.

Trial registration number: NA.

P-338 Endometriosis is associated with high-risk of ovarian, endometrial, cervical and thyroid cancer: a nationwide retrospective cohort study

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Study question: Is endometriosis associated with high-risk of cancers?

Summary answer: Endometriosis is associated with increasing risk of overall cancers especially with ovarian, endometrial, cervical, and thyroid cancer.

What is known already: The relationship between cancer and endometriosis was still unclear. Many reports suggested that endometriosis associated with increased risk of gynecological cancers, especially ovarian cancer but the conclusion was still inconsistent.

Study design, size, duration: A retrospective population-based cohort study was performed by linking to National Health Insurance Research Database (NHIRD) in Taiwan. A total of 19,378 women with newly identified endometriosis from 2000 to 2008 and 96,890 multivariable-matched controls (1:5) were selected.

Participants/materials, setting, methods: Sociodemographic and other potential confounding factors were selected as adjusted variables from database. They were compared between the endometriosis and control cohort using a Chi-square test. The Cox regression model adjusted for potential confounders was used to assess the risk of cancers due to endometriosis.

Main results and the role of chance: After adjusted potential confounders, the adjusted hazard ratio associated with endometriosis was 1.64 (95% confidence interval (CI) 1.49–1.81) for overall cancers, 4.77 (95% CI 3.40–6.70) for ovarian cancer, 4.19 (95% CI 2.91–6.04) for endometrial cancer, 2.67 (95% CI 1.93–3.70) for cervical cancers, and 1.64 (95% CI 1.15–2.35) for thyroid cancers.

Limitations, reasons for caution: Endometriosis may delay diagnosis by asymptomatic that lead to estimate the latent period of endometriosis and cancer occurring hardly and overestimate hazard ratio between cancer and endometriosis.

Wider implications of the findings: Estrogen stimulation and chronic inflammation might be as putative mechanisms to endometrial cancer. Besides, estrogen receptors participate in cellular processes contributing to enhanced mitogenic, migratory, and invasive properties of thyroid cells. The investigation of association between endometriosis and cancers would advance the exploration of endometriosis etiology.

Trial registration number: IRB permit number: CMU-REC-101-012.

P-339 The impact of endometriomas on blastocyst euploidy in cases undergoing comprehensive chromosomal screening (CCS) for recurrent implantation failure (RIF)

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Study question: Does the presence of endometrioma have an impact on CCS results of embryos in ICSI cycles using array comparative genomic hybridization (CGH)?

Summary answer: The rate of aneuploidy in embryos detected by aCGH does not differ in patients with endometriomas. However live birth rate is lower cases with endometriomas.

What is known already: The impact of endometrioma per se on IVF/ICSI outcome has long been evaluated by numerous groups and it is still controversial. Endometriomas can be suspected to have profound effects on oocyte/embryo quality as well as certain embryo development characteristics. It is reported that endometrioma/endometriosis, can adversely affect the structural integrity of the oocyte spindle, potentially resulting in aneuploidy.

Study design, size, duration: Retrospective case-control study conducted in CCS cycles with women aged under 38 treated in Bahceci Fulya IVF Centre between January 2013 and January 2015 and includes only patients with recurrent implantation failure. Two groups of patients were selected: group A patients with endometrioma and group B patients with unexplained infertility. Each group included only the cases with trofectoderm biopsy.

Participants/materials, setting, methods: A total of 23 patients in group A were matched to 53 patients in group B representing respectively 27 and 69 oocyte pick up procedures, 16 and 42 embryo transfer. Each patient underwent a controlled ovarian hyperstimulation and ICSI with frozen embryo transfer. Primary end-point was rate of euploid embryos and secondary end points were implantation rate, clinical pregnancy rate, spontaneous abortion rate.

Main results and the role of chance: Mean age, BMI and number of previous attempts were similar in group A and B ($33.1 \pm 3.6/33.1 \pm 3.0$; $22.6 \pm 2.4/23 \pm 3.1$; $4.2 \pm 1.2/4.3 \pm 2.6$)($p < 0.05$). Number of oocytes harvested, number of MII oocytes, fertilization rates were also similar in group A and B. ($13.9 \pm 7.1/15.1 \pm 10$; $10.8 \pm 5.9/11.5 \pm 8.4$; 80.1/77.6%). There was no significant difference in the number of euploid blastocysts between group A (25/81, 30.9%) and group B (61/163, 37.4%). Implantation rates and clinical pregnancy were found to be 38.1%, 43.7% for group A and 52% and 64.2% for group B embryos respectively ($p < 0.05$). In group A LBR was significantly lower than group B (18.8% vs. 47.6%).

Limitations, reasons for caution: Data were collected retrospectively using the database of our clinic. Sample size is relatively small but our study provides statistically significance that endometriomas decreases LBRs. Further larger series are needed to confirm these findings.
Wider implications of the findings: To our knowledge, this is the first study evaluating the blastocyst euploidy rates in cases with endometriomas. The study provides statistically significance that endometriomas decreases LBRs. This result may support decreased endometrial receptivity caused by endometriosis.
Trial registration number: None.

P-340 Role of epigenetic modification and expression of HOX genes in hypersensitivity of endometriosis and novel treatment approaches

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Study question: What is the cause of hypersensitivity in endometriosis and what are mechanisms of sensory nerve formation in eutopic or ectopic lesions?
Summary answer: Aberration in genes expression due to selective DNA methylation of gene involved in various aspect of sensory nerve formation, lead to pain sensation in endometriosis.
What is known already: Endometriosis is usually associated with infertility and pelvic pain such as chronic dysmenorrhea, intermenstrual abdominal and pelvic pain, back pain, dysuria and dyspareunia.

Nevertheless, no information is available on mechanisms of sensory nerve formation in eutopic or ectopic lesions. Since HOX genes have important roles in both reproductive tract and nerve growth, we decided to determine role of them in formation of new nerve fibers in endometriotic lesions.

At the other hand we designed another step as second phase of study to investigated epigenetic modification (DNA methylation) of promoter region of these genes.
Study design, size, duration: Samples obtained from fifteen patients (15 with and 15 without endometriosis) of reproductive age with normal menstrual cycles, where the same patient provided both eutopic and ectopic endometrium (endometriomas) and control samples were surgically checked for the absence of endometriosis.
Participants/materials, setting, methods: The expression profile of 84 genes of *HOX* family related to various aspects of nerve fibers formation (formation of nociceptors, dopaminergic neurons and signal transduction) was investigated using qRT-PCR array. Epigenetic modification (DNA methylation) assessed using Mecp2 antibody and qRT-PCR CHIP array technique. Informed consent was obtained from patients.

All measurements were performed in triplicates on independent biological replicates.
Main results and the role of chance: Our data showed significant over-expression of some genes which are involved in various aspect of development of sensory neurons in the tissue and signal transduction such as genes that involved in relay pain, touch and GABAergic neurons formation (VAX1, LBX1, LBX2 and PITX2), dopaminergic neurons (EN1 and PITX3), committed to a cutaneous sensory neuron fate (PAX3), chemosensory integration (PHOX2b), promote nerve formation (DRGX, EMX1, PHOX2B and OTP), various aspects of somatic motor neuron (ISL1, 2), neuronal migration (HLX) and touch/mechanosensory neurons (SHOX2) in euotopic and ectopic tissue versus control group. All of these genes have significant differences more than 30–1800 times in fold regulation between groups and *P* value for each of them is less than 0.001.

On the other hand, our data showed significant hypo-methylation of some genes which are involved in in relay pain, touch and GABAergic neurons formation (VAX1, LBX1, LBX2 and PITX2), dopaminergic neurons (EN1 and PITX3), committed to a cutaneous sensory neuron fate (PAX3), chemosensory integration (PHOX2b), promote nerve formation (DRGX, EMX1, PHOX2B and OTP), various aspects of somatic motor neuron (ISL1, 2), neuronal migration (HLX) and touch/mechanosensory neurons (SHOX2) in euotopic and ectopic tissue versus control group.
Limitations, reasons for caution: We investigated *HOX* genes expression profile in hypersensitivity of endometriosis, according to our finding; it seems that determining of another genes that may play a role in development of sensory neurons, could help to control of pain in endometriosis.
Wider implications of the findings: Due to epigenetic modification, overexpression (up to 1800 times) of some HOX family related genes that play critical roles in sensory, adrenergic and cholinergic neurons formation, lead to aberrant innervations of ectopic and euotopic tissues. therapeutic approach including novel methods (e.g., Using siRNA for gene silencing process) can be use.
Trial registration number: 13414.

P-341 Significant improvement in endometrial thickness with use of tamoxifen citrate in case with persistently thin endometrium: a retrospective study of 52 patients

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Study question: Does tamoxifen citrate increase endometrial thickness in women with earlier multiple cancelled embryo transfer cycles due to thin endometrium despite using estradiol valerate, pentoxifyllin, vitamin E, Sildenafil and granulocyte coloney stimulating factor ?
Summary answer: Use of tamoxifen results in significant improvement in endometrial thickness from 6.8 mm to more than 8 mm.
What is known already: Persistently thin endometrium is a very difficult entity to treat and is responsible for embryo transfer cancellation in a substantial number of women. The available modalities of treatment like high dose of estradiol valerate, sildenafil citrate, pentoxifyllin, vitamin E and granulocyte coloney stimulating factor have been useful in increasing the endometrial thickness only in few of these women. An earlier published small case series of 3 women with thin endometrium showed improvement in the thickness after using tamoxifen citrate.
Study design, size, duration: This is a historical case control study of 52 women with persistently thin endometrium with normal hysteroscopy and failed 2–3 cycle of estrogen replacement. All cases were recruited between February 2014 and November 2015. All these women had failed to achieve an adequate endometrium of 8 mm after using estradiol valerate 12 mg per day for atleast 21 days previously. In subsequent cycles, they failed to improve even with addition of above mentioned adjuvants.
Participants/materials, setting, methods: After initial failed attempt, they were given tamoxifen citrate 20 mg for 5 days from day 2 of periods. They underwent ultrasound for measuring follicle size and endometrial thickness from day 7/8 of periods. Cycles without dominant follicle were changed to estrogen replacement cycles by adding 8 mg of estradiol valerate. Ovulation day endometrial thickness was measured in case the follicular grew, or else, estradiol was continued for total of 21 days and endometrial thickness measured at repeated intervals.
Main results and the role of chance: 52 women were evaluated for 136 endometrial preparation cycles. Total 116 cycles using estradiol valerate, vaginal sidenafil citrate, oral pantoxiphylene, vitamin E and intrauterine instillation of G CSF were cancelled as mean endometrial thickness (ET) of these cycles was less than 6.8 mm. With addition of tamoxifen in the subsequent cycle, the ET showed significant improvement compared to the previous cycles. Of these, 43 women had improvement in endometrial thickness to > / = 8 mm, while for the remaining 9 women, endometrial thickness remained less than 6.9 mm. Out of these 43 women 35 women attained a mean of 9.8 mm thickness in first tamoxifen cycle while other 8 women reached mean endometrial thickness of 7.3 mm. In second tamoxifen cycle these women attained mean ET more than 8 mm. Overall, the biochemical pregnancy rate was 48% and clinical pregnancy rate was 39%.
Limitations, reasons for caution: It is a retrospective study with small sample size. The women acted as their own controls.
Wider implications of the findings: This study adds a new and potentially safer option for women with persistently thin endometrium. Further robust data in terms of large multicentric randomised controlled trials are needed to substantiate the findings since it will be difficult to get an adequately powered study in single centric study.
Trial registration number: Not applicable as it is a retrospective analysis.

P-342 Endometrial B-cell lymphoma 6 (BCL6) and Sirtuin 1 (SIRT1) Immunostaining are prognostic factors for implantation failure in the setting of ART

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Study question: Does BCL6 immunostaining predict pregnancy rate among patients with unexplained infertility after embryo transfer?
Summary answer: BCL6 immunostaining is highly prevalent in women with unexplained infertility (UI) and a strong predictor of IVF failure.
What is known already: We recently reported BCL6 over expression as a biomarker for endometriosis in the setting of otherwise unexplained infertility (UI). Increased BCL6 immunostaining is also observed in the baboon model of endometriosis by 9 months after disease induction, while reduced post-surgical BCL6 expression is associated with improved pregnancy rates. Involvement of BCL6 in chronic endometrial inflammation is suggested by endometrial overexpression in chronic endometritis in the bovine model. BCL6 protein along with its binding partner, SIRT1, acting via hedgehog family proteins (e.g., Gli1) is a candidate mediator driving progesterone resistance and subfertility seen in patients with endometriosis.
Study design, size, duration: BCL6 and SIRT1 immunostaining of prospectively collected, mid-secretory endometrial samples was evaluated using HSCORE by a single observer, blinded to clinical information. Pregnancy outcomes were examined in 66 UI subjects undergoing an endometrial biopsy within 6 months prior to a single embryo transfer cycle. Comparison between medians utilized Mann-Whitney test and comparison of pregnancy rates utilized Fisher's exact test.
Participants/materials, setting, methods: Mid-secretory phase was defined as urine LH surge plus 6–10 days. Exclusion criteria included Age >40, BMI ≥30, use of medications known to affect reproductive hormones during the previous 3 months, known reproductive tract abnormalities, and endometrial dating >2 days discrepancy from expected histology. Immunostaining was performed using a monoclonal antibody on an automated system. The BCL6 and SIRT1 HSCORE cutoffs were based on previously reported and unpublished evaluation as endometriosis biomarkers.
Main results and the role of chance: Pregnancy occurred in 10/15 (66.7%) subjects with UI and negative BCL6 immunostaining as compared to 7/51 (13.7%) subjects with UI and positive staining ($p < 0.001$). SIRT1 staining was evaluated in a subset of subjects. Pregnancy occurred in 6/12 (50%) subjects with UI and negative SIRT1 staining as compared to 4/29 (13.8%) subjects with UI and positive staining ($p = 0.040$). Western blot immunostaining of an independent set of samples from controls and women with UI ($n = 33$) demonstrated a linear correlation coefficient of 0.653 ($p < 0.001$) and double immunofluorescence co-localized BCL6 and SIRT1 in the nuclei of women with UI, suggesting that known binding partners, BCL6 and SIRT1, may act together in an important pathway for endometrial dysfunction.

The high rates of BCL6 and SIRT1 overexpression in the UI population studied suggest that endometrial dysfunction is common in UI. The large difference in pregnancy rates for those positive and negative for BCL6 and SIRT1 immunostaining, suggest that these biomarkers may be a useful prognostic biomarker for endometrial dysfunction.
Limitations, reasons for caution: A small proportion of subjects with UI stained negative for BCL6 and SIRT1, limiting the statistical power of the pregnancy rate comparison. Many of the biopsied subjects with UI had previously undergone failed cycles, potentially skewing the UI population heavily toward those with endometrial dysfunction.
Wider implications of the findings: If confirmed by larger prospective studies, endometrial expression of BCL6/SIRT1 and related biomarkers could be a useful test to predict endometrial dysfunction in infertility patients as well as allow development of individualized and targeted therapies for implantation failure.
Trial registration number: Not applicable.

P-343 Identifying a subgroup of endometriosis patients with potential high risk for Endometriosis associated cancers

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Study question: Is it possible to identify high risk women for Endometriosis Associated Cancer (EAC), based on cancer stem cell markers in their endometrium?
Summary answer: We could identify a subset of endometriosis women expressing aberrant levels of pre-malignant, epithelial-mesenchymal-transition (EMT) and cancer stem cell (CSC) markers in endometrium.
What is known already: Epidemiological studies show that a subset of women (0.7–2.5%) with endometriosis have higher risk for ovarian endometrioid and clear cell ovarian carcinoma. Now, it is very well known that endometrial stem cells (EnSC) are actively involved in endometrial regeneration and also are present in endometrioma. Though the mechanism is not clearly understood, a variety of molecular events have been postulated with the malignant transformation of endometriotic tissue. These include change in the expression of TP53, BCL-2, PTEN, ARID1A, CTNNB1.
Study design, size, duration: Biopsies were collected from endometrium ($n = 18$; P-EnSC) and endometrioma ($n = 11$; P-EndoSC) from patients undergoing surgery for endometriotic cyst. Patients who were not under hormonal treatment or carrying any intrauterine device for at least three months prior to surgery were enrolled. Endometrium was collection from a group of healthy fertile women ($n = 17$; H-EnSC). Biopsies were enzymatically digested and later EnSC and EndoSC populations were sorted with flow cytometry using markers CD90 CD73 and CD105.
Participants/materials, setting, methods: Customised gene expression assay were performed for panel of 40 genes belonging to the families of oncogenes, tumor suppressors, EMT, CSC, pluripotency, proliferation, anti-apoptosis, hormonal markers. Variability between patients for molecular expression were assessed by univariate PCA and multivariate OPLS-DA modelling (SIMCA 14 software). Molecular deregulation among patients were assessed by IPA. Invasion of P-EnSC, EndoSC in response to Paclitaxel and Cisplatin treatment were assessed using 3D spheroid invasion assay.
Main results and the role of chance: Real time PCR analysis showed a downregulated trend in tumor suppressor genes TP53/p53, ARID1A and upregulated trend in CD133/PROM1 and SMO expression. Based on heat map analysis of cancer stem cell relevant molecular expression in EnSC and EndoSC, we identified three patients as potentially high-risk' for EAC and named them as P-EnSC-hi or P-EndoSC-hi. The rest of the patients within each groups were called as low risk patients (P-EnSC-lo and P-EndoSC-lo) for EOC. Both P-EnSC-high-risk' and P-EndoSC- high-risk' had overexpression of pre-malignant genes TGF-β, TP53, FOS; CSC and EMT genes ER-α, SNAI1, KRAS, CTNNB1, NOTCH3 and EMT and most importantly ARID1A, previously shown to have strong association with EAOC. Moreover, trend curves with statistically significant genes within these subgroups revealed "low-risk" patients with gene expression pattern similar to H-EnSC, while high-risk' subgroup presented an expression trend more closely to the cancer profile similar to endometrial (Ishikawa) and ovarian cancer lines (SKOV3 and A2780). Functional assessment of subgroup risk status by invasion area in response to drug treatments by10 nM Paclitaxel and 10 μM cisplatin showed P-EnSC-hi subgroup with less invasion and more chemo-sensitivity compsring P-EnSC-lo, indicating role of P-EnSC in tumor progression (FC: –8.22, CI: 2.768 to 13.69, $P < 0.01$).
Limitations, reasons for caution: We included only endometriosis patients without any hormonal treatment. Although this improves the quality of the results, it limits the overall sample size. Though we tried to adopt a physiologically relevant *in vitro* model, an *in vivo* approach of testing tumorogenesis would have led to better understanding of EAC.
Wider implications of the findings: This study for the first time shows the possibility to identify women with endometriosis having high risk for endometriosis associated cancer (EAC), based on the molecular expression we have identified. This may potentially help in identifying patients with high risk for EAC and offer prophylactic measures to reduce EAC.
Trial registration number: NA.

P-344 Compartmentalized Gene Expression profile of receptive endometrium, ENPP3- a Novel biomarker for progesterone regulated endometrial receptivity

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Study question: Identify potential progesterone regulated endometrial receptivity biomarker.
Summary answer: Progesterone regulated N-glycated Ectonucleotide pyrophosphatase/phosphodiesterase 3 (ENPP3) can potentially serve as biomarker for endometrial receptivity.
What is known already: With recent advances in omics technology, several studies were attempted to elucidate the gene expression of progesterone modulated receptive endometrium, but no unequivocal receptivity marker is yet defined in humans. We also lack a molecular marker that could be used to assess endometrial receptivity prior to embryo transfer. Such a biomarker if available, would benefit to assay the women undergoing IVF treatment, women with recurrent implantation failure and various endometrial receptivity disorders.
Study design, size, duration: Receptive ($n = 8$) and non-receptive ($n = 8$) endometrial biopsies were obtained during LH+7. Pure stromal and epithelial cells were isolated by Laser-capture microdissection (LCMD) and gene expression was studied by microarray, reconfirmed by real time PCR and immunohistochemistry. Expression of ENPP3 was analyzed in uterine fluid collected from receptive ($n = 6$) and non-receptive ($n = 6$) women by western blotting. *In vitro* functional assay with 3-dimension cell cultures was done to see the role of ENPP3 in human embryo implantation.
Participants/materials, setting, methods: Receptive and non-receptive (with 200 mg mifepristone on LH+2) endometrial biopsies were obtained from proven fertile women. Stromal and epithelial cells were dissected by LCM and gene expression studied by microarray (Affymetrix HuGene v1.0) and analysed by Ingenuity Pathway Analysis. Protein expression was studied by immunohistochemistry. Uterine fluids and endometrial tissue lysates were analyzed for the expression of glycated-ENPP3 by Western blot. Blastocysts were acquired through standard IVF or ICSI for 3dimensional cell cultures.
Main results and the role of chance: Top canonical pathway obtained on bioinformatics analysis is NRF2 mediated oxidative stress response and upstream regulators included transcription factor CBX5 (activated), CSF2 and EBF1 (inhibited). Microarray findings were validated by real time PCR and immunohistochemistry and were found to be in concordant with the microarray study, the expression of Metallothioneins (MT1G and MT2A) and ENPP3 were significantly down-regulated in both stromal and glands, whereas SFRP4 upregulated.

Very interestingly, both the protein and mRNA for ENPP3 expression showed significant down-regulation in epithelial compartment of treatment group ($p < 0.01$). No stromal immunostaining of ENPP3 was seen in either control or treatment group. Cyclical expression, highest in mid-secretory and lowest in proliferative phase ($p < 0.01$) was observed for ENPP3. N-Glycosylated ENPP3 was observed both in uterine fluid and endometrial tissue lysates. Depletion of progesterone down regulated its expression ($p < 0.01$). *In vitro* functional assay using 3-dimensional cell cultures confirmed the receptivity of the endometrial construct falling in line with the expression of ENPP3 ($p < 0.01$).

Paired *T*-Test and SAM methods were used for analysis of microarray data. Mann-Whitney's test was applied for immunohistochemistry and Western blot analysis.
Limitations, reasons for caution: Validation of the result with ENPP3 in a clinical set-up is required before extending it for clinical proposes. The physiological role of ENPP3 in endometrial receptivity and uterine fluid need to be investigated.
Wider implications of the findings: ENPP3 may offer a diagnostic tool for endometrial receptivity in women, especially in IVF before planning embryo transfer. There is also a possibility to develop an inhibitor or molecule that could alter the function of ENPP3 and thus use it for fertility control.
Trial registration number: NA.

P-345 Does low dose r – FSH ovarian stimulation negotiate thin endometrium problem in oocyte donation program?

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Study question: To evaluate whether low dose ovarian stimulation enhances estrogen receptivity and endometrial thickness better than exogenous estrogen supplementation for successful embryo implantation in oocyte donation progrmmes.
Summary answer: Administration of low dose r-FSH enhances endometrial thickness and gradation resulting in improved receptivity required for successful implantation of ensuing blastocyst.
What is known already: It is generally accepted that thin endometrium (<7 mm) observed on ultrasound results in lower implantation rates in IVF procedure probably due to obvious reason of abnormality in estrogen receptors on the endometrium. Embryo implantation, clinical and ongoing pregnancy rates are significantly higher in patients with an endometrial thickness >9–10 mm. The target cross-section endometrial thickness is >7 mm, with a triple-line endometrial pattern (grade), whereas endometrial thickness <6 mm is associated with a decreased probability of achieving a full-term pregnancy. Ultrasound monitoring of endometrial development plays a key role in steroid replacement for donor egg programs or frozen embryo transfer.
Study design, size, duration: Out of 167 women undergoing oocyte donation program during January 2014 to July 2015, 67 women with thin endometrium (<7 mm on day 14) did not achieve pregnancy. Blastocyst transfer was done despite thin endometrium since pregnancies in endometrium <3.7 mm have been reported. These 67 women were subjected to minimal stimulation protocol of 50–75 IU of r–FSH in the next cycle. Blastocyst transfer was done in 63 of these 67 women.
Participants/materials, setting, methods: 67 women undergoing their second oocyte donation cycle with Protocol B involving minimal stimulation with r–FSH were compared with their own previous cycle involving standard estrogen supplementation from day 2 alongwith added sildenafil from day 5 (protocol A). All women, whether undergoing protocolA/B, received luteal phase support from day 14. First cycle involved fresh blastocyst transfer whereas Frozen–thawed blastocyst transfer was done in second cycle.

TVS monitoring of endometrial thickness and gradation was done serially from day 14 to day ET.
Main results and the role of chance: Endometrial thickness on day 14 in women undergoing Protocol B was significantly higher as compared to those undergoing Protocol A (8.0 ± 2.0 mm vs. 5.3 ± 1.5; $p = 0.0015$). The endometrial echo-pattern in group B was also significantly enhanced (3.1 ± 0.32) as against that in group A (2.4 ± 0.24; $p = 0.0328$). Similarly, Day 18 monitoring of endometrial response showed extremely significant difference in endometrial thickness (9.2 ± 2.0 mm vs. 6.1 ± 1.4 mm; $p = 0.0001$) as well as echo-pattern/grade of endometrium (3.7 ± 0.4 vs. $2.\ 7 \pm 0.4$; $p = 0.002$) when protocol B was followed. Finally, on the day of blastocyst transfer too, the endometrial response showed a rising trend in endometrial thickness and gradation (1.1 ± 1.05 mm, pattern 3.9 ± 0.2) in Protocol B compared to protocol A women (8.0 ± 2.0 mm; 3.1 ± 0.5) respectively. Whereas no pregnancy resulted in any of the 67 women in their first cycle with Protocol A; out of the 63 women who had blastocyst transfer in their second cycle with protocol B, 15 resulted in clinical pregnancies (Pregnancy rate 23.09%) and 2 were ectopic pregnancies.
Limitations, reasons for caution: This is the first study employing minimal stimulation protocol in patients with thin endometrium undergoing oocyte donation programs. Although this study has a small sample size, yet it cannot be compared with studies that include adjuvents and intrauterine perfusions with cytokines to improve endometrial thickness. Therefore, large multicentric study is warranted.

Wider implications of the findings: Thin endometrium is a very difficult problem to deal with for clinicians world over. Administration of low dose r-FSH may aid in tiding over the problem of thin endometrium to some extent, thus improving clinical pregnancy rates in oocyte donation programmes as observed in this study.
Trial registration number: Not Applicable.

P-346 Endometrium co-culture, 3D endometrium co-culture, 3D polymeric carrier aided endometrium co-culture: comparative effects on implantation

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Study question: Can 3D endometrial co-culture systems composed of or coated with extracellular matrix components and endometrial cells increase adhesion and invasion of blastocysts?
Summary answer: 3D culture systems and 3D Polymeric cell carriers coated with appropriate cell and/or extracellular matrix components can aid blastocyst adherence and invasion.
What is known already: In spontaneous pregnancies, the rate of implantation failure in first trial is 25–40%. In ART cases maximum implantation rate was shown to be 40–60% in the metaanalyses. It was well confirmed that each good quality embryo can not implant in every cycle.

The cross talk between the hatched blastocyst and the endometrium can only occur in a very short period- the window of implantation'. Implantation occurs in three phases: apposition/attachment-adherence/penetration.

This biological event is driven by a complicated mechanism. Most of the *in-vitro* co-culture methods do not support invasion of the blastocysts and only adherence can be maintained to some extent®.
Study design, size, duration: 1. routine endometrial co-culture.

2. three-dimensional culture of mouse endometrium epithelial and stromal cells on Matrigel®.

3. polymeric sponge coated by collagen and endometrial epithelial and stromal cells.

Experiments were repeated three times.

Implantation parameters were evaluated by IF and by WB and qRTPCR.

Invasion depth was evaluated by laser scanning confocal microscope and SEM.

Data was quantified by related softwares for statistical analysis.
Participants/materials, setting, methods: Superovulated hybrid female and male (Balb/c) mice were used to obtain blastocysts (+endometrium).
A. **Blastocysts placed on:** 1. Endometrial epithelial+stromal cells together.

2. Stromal cells **inside** matrigel +epithelial cells **over** matrigel.

3. Endometrial cells on the **collagen** coated **poly (L-lactic acid-co-D, L-lactic acid) polymeric** carrier.
B. At hours 24, 48, 72, 96-evaluation for progesterone receptors and implantation markers.

1. Fixation for IF and SEM.
2. WB.
3. qRTPCR.

C. Evaluation of invasion and surface by LS confocal microscope and SEM
Main results and the role of chance: 1. Endometrial co-culture: -Adhesion of blasts: very early.

- Increase of E-cadherin and L-selectin: 24 h after incubation. E-cadherin expression did not decrease with incubation time.
- No progesteron receptor activity was seen after 24,48,72 h. At hour 96, the result was suspicious.
- No definitive decidual area formation after 24 h but a mild change in some multiadhesive glycoproteins was seen after 72 h. It may also be related to increase of fibroblast-like cells in the culture environment.
- No definite invasion sign.

2. Endometrial co-culture with Matrigel: -Adhesion: more intense compared to first group. Increase of E-cadherin and L-selectin.

- Occasional progesterone receptors on the stromal and epithelial cells. Mild increase with incubation.
- Increase of laminin and fibronectin in the area under the blastocyst, suggesting a probable decidual zone.
- Invasion under the blastocyst area accompanied with decrease in E-cadherin.
- Mild increase in LIF and Hox expression.
- Pinopode formation.

3. Endometrial co-culture with collagen coated polymeric foam: -Intense adhesion: Higher increase of E-cadherin and L-Selectin, at early phases.

- Progesterone receptors on endometrial cells.
- Increase of laminin and fibronectin.
- Invasion-accompanied with decrease in E-cadherin.
- Increase of LIF and Hox expression.
- Intense pinopode formation.

WB and qRTPCR results supported the semiquantitative findings by these first results.
Limitations, reasons for caution: 1. 1. These are the results of first trial, two repetitions will be completed.

2. In polymeric carrier group most implantation parameters are better, but invasion depth was shorter. This is probably caused by the hardly degradable property of the polymer. A foam composed totally of collagen will be tried.
Wider implications of the findings: 1. Here we propose two very effective *in-vitro* implantation models. Both of them can be used to see the *in-vitro* development of the blastocyst together with the newly developing city and syncythiotrophoblasts.

2. Polymeric cell carrier can be developed to be used in embryo transfers during ART cycles.
Trial registration number: This is not a clinical trial.

P-347 Enhanced endometrial receptivity and augmented live birth rate in IVF cycles is favoured by Low IL-6 levels in follicular fluid

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Study question: To correlate levels of IL-6 in follicular fluid with endometrial thickness on day of embryo transfer and subsequently study its effect on live birth rates.
Summary answer: Lower FF IL-6 level correlates with enhanced endometrial receptivity favourable for implantation and increased live birth rate in IVF cycles.
What is known already: IL-6 is known to be a pleiotrophic cytokine with multiple cellular effects. Measurements of serum and follicular fluid (FF) levels of IL-6 in women undergoing *in vitro* fertilization (IVF) have been shown previously to be associated with etiology of infertility, stimulation protocol, fertilization rates and pregnancy outcome.
Study design, size, duration: Retrospective analysis of data from non-PCOS, non-endometriotic women undergoing antagonist IVF cycles (n = 133) at our infertility centre from November 2014 to June 2015. Cycles were divided into Low (≤47.5 pg/ml) and High (>47.5 pg/ml) FF IL-6 groups.
Participants/materials, setting, methods: FF IL-6 levels were estimated in each cycle from FF pooled from follicles from which an oocyte had been obtained. After evaluation of fertilization and embryo development to blastocyst stage, D5 blastocyst transfer was done. Endometrial thickness measured in cm using TVS on day of embryo transfer and live birth rate were the main outcome measures.

Main results and the role of chance: Overall live birth rate was 28.57% (38/133). Low FF IL-6 group (n = 100) had significantly higher endometrial thickness (1.19 ± 0.02 vs. 1.04 ± 0.03 cm, p = 0.0053) and live birth rate {32% (32/100) vs. 18.18% (6/33); $p < 0.0001$} on day of embryo transfer than high FF IL-6 group (n = 33). FF-IL-6 levels correlated inversely with endometrial thickness on day of embryo transfer (Pearson r = –0.23, p = 0.0097). Lower Follicular fluid level of IL-6 on day of oocyte aspiration thus facilitates development of a good endometrium receptive for the incoming embryo to implant. This is clearly reflected in the significantly higher live birth rate observed in the low FF IL-6 group.
Limitations, reasons for caution: This study measured IL-6 levels in follicular fluid pooled from all follicles in each patient. Although tedious and time consuming, it would be more befitting to measure the levels in individual fluid aspirated from each follicle. Also, multicentric trials are required to ascertain IL-6 as a marker of endometrial receptivity.
Wider implications of the findings: Follicular fluid level of IL-6 may be an early indicator of developing endometrium having potential for successful embryo implantation.
Trial registration number: Not Applicable.

P-348 Frozen-thawed embryo transfers in natural cycles with spontaneous ovulation are associated with increased clinical pregnancy rates

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Study question: Among patients undergoing frozen embryo transfers (FET), are natural cycles (NC) associated with better clinical pregnancy rates (CPR), when compared to human chorionic gonadotropin (hCG)-triggered cycles?
Summary answer: NC without luteal phase support (LPS) are associated with the highest CPR, followed by NC with LPS (NC+LPS) and hCG-triggered FET with LPS (hCG+LPS).
What is known already: While some studies have associated NC with spontaneous ovulation with higher CPR, when compared to hCG-triggered cycles, the evidence on this topic is conflicting and information of the additional effect of LPS is lacking.
Study design, size, duration: This retrospective study included all (n = 2896) consecutive FETs of vitrified cleavage and blastocyst stage embryos warmed between January 2010 and April 2015 in a tertiary centre. The FETs were grouped by type as follows: NC (n = 1063), NC+LPS (n = 570) or hCG+LPS (n = 1263).
Participants/materials, setting, methods: We performed mixed-effect multilevel multivariable regression analysis to account for the clustering of FETs using embryos derived from the same patient and/or ovarian stimulation cycle. Adjustment for the following potential confounders was also performed: female age at oocyte retrieval, body mass index (BMI), number of oocytes retrieved, fresh cycle pregnancy outcome, embryo transfer rank, number of embryos transferred, embryo stage and grade, and endometrial thickness. Bonferroni adjustment for multiple comparisons was performed whenever indicated.
Main results and the role of chance: The CPR/FET was significantly higher in the NC group (44.6%) as compared with the NC+LPS (36.8%, p = 0.007) and hCG+LPS groups (26.4%, $p < 0.001$). The lower performance in terms of CPR in the hCG+LPS remained significant even after adjusting for potential confounding using multivariable regression analysis [adjusted odds ratio (95% confidence interval) compared to the hCG+LPS group: 2.24 (1.73–2.91) and 1.67 (1.35–2.06) for the NC and NC+LPS groups, respectively].

A sensitivity analysis restricting the sample only to the first FET performed by the couple in our centre was also performed. The predicted CPR in the multivariable logistic regression analysis were still significantly higher in the NC (52.8%) and NC+LPS (42.5%) groups when compared to hCG+LPS (31.6%, all Bonferroni-corrected pairwise comparisons with $p < 0.01$).
Limitations, reasons for caution: The interpretation of the findings of this study is limited by the retrospective nature of the analysis and the potential for unmeasured confounding.
Wider implications of the findings: While facilitating the endocrine monitoring of FETs, hCG ovulation triggering and the use of LPS in a NC should be reconsidered, in light of the potential negative effect on the pregnancy outcome.
Trial registration number: None.

P-349 Altered metabolic pathways in endometrial cells treated with mycobacterial heat shock protein HSP65 causes aberrant decidualization

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Abstract withdrawn by the authors

P-350 Combined mRNA microarray and proteomic analysis of endometrium of women with repeated implantation failure (RIF) and fertile controls

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Study question: Is there a difference in gene and protein expressions in the endometrium between women with RIF and fertile control during window of implantation?
Summary answer: In RIF patients, gene analysis revealed 6 down-regulated genes, 9 up-regulated genes, while differentially expressed proteins exhibited 145 down-regulation, 129 up-regulation compared to fertile controls.
What is known already: Implantation failure remains an unsolved obstacle in reproductive medicine and is a major cause of infertility in otherwise healthy women. Inadequate uterine receptivity is responsible for approximately two-thirds of implantation failures, whereas the embryo itself accounts for only one-third of failures. Therefore, the management of repeated implantation failure (RIF) is one of the most difficult issues in assisted reproduction. We performed global gene profiling, using microarray technology, of endometrium during the window of implantation for patients with RIF versus fertile controls. In addition, we have pursued validation of gene expressions by proteomic analysis.
Study design, size, duration: In this prospective cohort study, mRNA and protein fractions were extracted from 45 endometrial biopsies obtained from women with repeated implantation failure (RIF) (n = 24) or fertile controls (n = 21) during the window of implantation (LH+7 to LH+10). Endometrial biopsies were collected using a Pipelle. Total RNA was isolated from endometrium tissue samples.
Participants/materials, setting, methods: mRNA and protein fractions were extracted from 45 endometrial biopsies obtained from women attending the infertility and gynecology clinics of an university hospital with repeated implantation failure (RIF) (n = 24) or fertile controls (n = 21) during the window of implantation (LH+7 to LH+10) and analyzed using microarray and LC-MS/MS in university genomic and proteomic laboratories, respectively. RIF patients had undergone ≥3 IVF failures; fertile controls had a history of ≥1 live birth.

Main results and the role of chance: In this study, we have applied a genome-wide transcript-level changes approach using oligo microarrays representing 37,600 genes, in order to define the genome profile of RIF patients as compared with fertile controls. Gene-array analysis revealed 15 genes exhibiting modified expression levels in RIF patients. Of these, 6 genes (40%) were down-regulated and only 9 genes (60%) were up-regulated. Classification of the down and up regulated genes to biological pathways revealed EIF2 signaling, acute phase response signaling, LXR/RXR activation, regulation of eIF4 and p70S6K signaling and FXR/RXR activation pathways. Proteomics analysis yielded the identification of 1531 proteins and 274 of these were calculated to be statistically significant ($p < 0.05$, fold change >40%). Differentially expressed proteins in RIF patients exhibited 145 down-regulation and 129 up-regulation.
Limitations, reasons for caution: The study model needs validation in larger study populations.
Wider implications of the findings: mRNA expression and proteomic analysis of endometrium differed in women with RIF and fertile controls. Such genomic and proteomic analyses could be used to allow a better understanding of the endometrial receptivity and the development of semi-invasive diagnostic tests for detecting endometrial receptivity.
Trial registration number: N/A.

P-351 NMR based metabonomics for identification of serum markers in women with dormant genital tuberculosis

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Study question: Can NMR based serum metabolic profiling discriminate dormant genital tuberculosis (GTB) women from controls?
Summary answer: Metabolites including 3-hydroxybutyrate, succinate, citrate, acetate, L-glutamine, L-lysine, L-arginine, L-proline and L-threonine hold promise as potential candidate markers for detection of sub-clinical GTB women.
What is known already: NMR based metabolic profiling has emerged as an useful tool for identification of biomarkers in biological fluids. It is reported that *Mycobacterium tuberculosis* in the endometrium significantly alters the energy metabolism and amino acid biosynthesis in dormant GTB cases. Also, dormant GTB impairs the receptive status of the endometrium leading to infertility.
Study design, size, duration: Women with dormant GTB ($n = 26$) and unexplained infertile women with repeated IVF failure as controls ($n = 26$) were included in the study. Serum samples were collected from these patients at the tertiary infertility care center during the period September 2011–March 2013.
Participants/materials, setting, methods: Unexplained infertile women having at least 3 IVF failure with and without dormant GTB were recruited for this study. 700 MHz proton NMR spectra of serum were recorded for both the groups. Multivariate analysis including principal component analysis, partial least squares discriminant analysis and orthogonal projection to latent structure-discriminant analysis was applied to all the spectra. Multiple correlation analysis of endometrial tissue and serum metabolites was performed.
Main results and the role of chance: A clear metabolic differentiation between women with dormant GTB and controls was observed. Metabolites including 3-hydroxybutyrate, succinate, citrate, acetate, L-glutamine, L-lysine, L-arginine, L-proline and L-threonine were found to significantly upregulated in serum of women with dormant GTB compared with controls ($p < 0.05$). Pearson's correlation analysis showed a significant positive (0.317–0.652, $p < 0.05$) and negative correlation –0.261to –0.619, $p < 0.05$) between the expression of endometrial tissue metabolites and serum metabolites.
Limitations, reasons for caution: Dormant GTB diagnosis with conventional techniques including culture, smear and PCR remains a challenge, owing to the paucibacillary nature of the tubercular bacilli and false positivity and negativity. Intra- and inter-individual variability, impact of diet and medication, population stratification should be minimized to identify putative metabolite markers.
Wider implications of the findings: The set of metabolite markers identified may be explored for diagnosis of dormant GTB which would eventually help clinicians in early management.
Trial registration number: None.

P-352 Endometrial expression of Leukemia Inhibitory Factor (LIF), LIF-Receptor and HOXA-11 but not HOXA-10 is significantly impaired in women with unexplained infertility during implantation window

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Study question: Is endometrial expression of HOXA-10, HOXA-11, LIF and LIF-R significantly different between fertile and infertile as well as within sub-groups of infertility during implantation window?
Summary answer: Endometrial expression of LIF, LIF-R and HOXA-11 but not HOXA-10 is significantly impaired in women with unexplained infertility during implantation window.
What is known already: HOXA-10, HOXA-11 and Leukemia Inhibitory Factor (LIF) with its receptor (LIF-R) have been indicated to play a significant role for the process of adhesion, embedment and implantation of blastocyst. Nevertheless, the possible relationship between their endometrial expression and infertility has not yet been clarified. Furthermore, no conclusions have been made regarding the expression patterns of HOXA-10, HOXA-11, LIF and LIF-R in the various sub-fertility groups during the implantation window.
Study design, size, duration: A prospective observational study was initiated on March 2013 at IAKENTRO, Infertility Treatment Center, Thessaloniki and 3rd Department of Obstetrics and Gynecology, Aristotle University of Thessaloniki. The present manuscript reports the results obtained until December 2015.
Participants/materials, setting, methods: Women having delivered at least one alive newborn were the study's control group (fertile women, group 1) while infertile women the patients' group (infertile women, group 2). An endometrial biopsy was obtained on 7^{th}–8^{th} postovulatory day. Expression of HOXA-10, HOXA-11, LIF and LIF-R was assessed in both epithelial and stromal cells by immunohistochemistry. Primary outcome was h-score. Parameters were compared between fertile and infertile women. A sub-analysis of expression was performed for various sub-fertility categories.
Main results and the role of chance: There were overall 90 women (25 fertile and 65 infertile) meeting the inclusion criteria. Endometrial tissue was obtained by 20 fertile and 55 infertile, of which 22 women were diagnosed with poor ovarian reserve, 13 with tubal infertility, 5 with endometriosis and 15 with unexplained infertility. Mean age was 31.1 ± 4.8 years for group 1 and 37.5 ± 3.8 for group 2 ($P = 0.001$). LIF and LIF-R h-score was significantly decreased in epithelial cells of infertile women compared with fertile controls ($P = 0.05$ and $P = 0.006$, respectively). No significant difference was observed regarding HOXA-10 and HOXA-11 epithelial endometrial expression as well as stromal expression of all molecules between two groups. LIF-R endometrial expression was found to be impaired in all sub-groups of infertility. Besides, LIF, LIF-R and HOXA-11 epithelial h-score presented their lowest values in women with unexplained infertility. Specifically, LIF epithelial h-score was 0.4 ± 0.2, LIF-R epithelial h-score 1.4 ± 0.2 and HOXA-11 epithelial h-score 0.1 ± 0.05 in women with unexplained infertility, the difference being significantly different with fertile controls ($P = 0.02$, $P = 0.03$ and $P = 0.005$ respectively).
Limitations, reasons for caution: Results presented in the abstract are based only on immunohistochemistry without having performed real-time PCR. However, this is the first study to report on expression levels of these molecules in the various sub-groups of infertility.
Wider implications of the findings: Our results indicate the significant role of HOXA-11, LIF and LIF-R in the aitiopathogenetic drawback complex of

unexplained infertility. As LIF-R epithelial expression was found to be impaired in all sub-groups of infertility, LIF-R seems to have a basic role in reassuring fertility along with a normal implantation process.
Trial registration number: The present trial has been approved by Institutional Review Board and Ethical Committee of AUTH.

P-353 3D Ultrasound assessment of effect of controlled ovarian stimulation on endometrioma volume

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Study question: Do endometriomas grow during controlled ovarian stimulation (COS) for ART?
Summary answer: Endometrioma volume increases during COS.
What is known already: Mean diameter of endometriomas before and after COS was compared in only one prior study and they were found similar.
Study design, size, duration: Women with endometriomas who underwent COS and oocyte pick –up were prospectively recruited over one year. An a priori sample size calculation revealed that 14 women would be required to detect a clinically significant change in endometrioma from a preset volume. Institutional ethics committee approved the protocol and each women provided informed consent.
Participants/materials, setting, methods: Twenty – five women with 28 endometriomas were recruited. Each underwent 3D ultrasound scan with Voluson E8 using sono automated volume calculation (SonoAVC) software. Endometrioma volume was measured at the first day of gonadotropin injection (V1) and on the day of ovulation trigger (V2). Volumes were compared with related samples Wilcoxon signed rank test.
Main results and the role of chance: Mean age (SD) was 36.1 (4.6) years. Median body mass index (25th–75th percentile) was 22.9 (19.3–25.7) kg/m^2, antral follicle count was 5 (3–8), and serum anti-Müllerian hormone level was 1.11 (0.7–1.83) ng/ml. Nine (36%) women were stimulated in a GnRH antagonist protocol, 13 (52%) in a long, and 3 (12%) in an ultralong GnRH agonist protocol,. Mean duration of stimulation was 10.3 (2.3) days with median total gonadotropin dose of 4500 (3113–4950) IU/day. Median number of follicles >14 mm was 4 (2–7), number of cumulus oocyte complexes was 5 (4–9), and metaphase-two oocytes was 4 (3–7). None of the endometriomas were punctured during oocyte pick-up. One patient developed an ovarian abscess after the procedure. Median V1 was 22.2 ml (12–30 ml) and median V2 was 24.99 ml (11.2–37.4 ml) with a p value of 0.001. Twenty-three out of 28 endometriomas (82%) grew to some extent during COS.
Limitations, reasons for caution: The study was adequately powered. A single operator who had experience with SonoAVC performed all the scans. SonoAVC, like all volume acquisition software, requires manual post-processing, which in theory could have biased the operator.
Wider implications of the findings: Even though the 3 ml average growth was statistically significant, it could be regarded clinically insignificant. This growth may have not been noticed in the previous study due to the notorious variability of diameter measurements, which is an imperfect surrogate of volume in this setting.
Trial registration number: None applicable. Local ethics committee approved.

P-354 Endometrial expression of progesterone receptors but not avb3 integrins is significantly decreased in women with unexplained infertility and poor ovarian reserve

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Study question: Is endometrial expression of progesterone receptors and avb3 integrins significantly different between fertile and infertile as well as within sub-groups of infertility during implantation window?
Summary answer: Progesterone receptors' but not integrins' expression is significantly decreased in epithelial cells of infertile women with unexplained infertility and poor ovarian reserve.
What is known already: Progesterone receptors type A and B as well as integrins avb3 are enrolled in the normal implantation procedure of blastocyst. Especially for progesterone receptors, the observed down-regulation during mid-luteal phase is essential to reassure endometrial receptivity. No definite conclusions, however, have been made about the expression patterns of these implantation mediators in the various sub-groups of infertility both in epithelial and stromal cells during implantation window.
Study design, size, duration: A prospective observational study was initiated on March 2013 at IAKENTRO, Infertility Treatment Center, Thessaloniki and 3rd Department of Obstetrics and Gynecology, Aristotle University of Thessaloniki. The present manuscript reports the results obtained until December 2015.
Participants/materials, setting, methods: Women having delivered at least one alive newborn during the last year consisted the control group (group 1) while infertile patients' group (group 2). Infertile were also categorized to those with ovarian failure (group 2a), tubal factor (group 2b), endometriosis (2c) or unexplained infertility (group 2d). Endometrial biopsy was obtained by Pipelle on 7th and 8th postovulatory day. Total progesterone receptors' (TPR), type-B receptors' (PR-B) and integrins' avb3 expression was evaluated by immunohistochemistry. Primary outcome was h-score.
Main results and the role of chance: There were overall 90 women (25 fertile and 65 infertile) meeting the inclusion criteria, of which endometrial tissue was obtained by 20 fertile and 55 infertile. There were 22 women diagnosed with poor ovarian reserve, 13 with tubal infertility, 5 with endometriosis and 15 with unexplained infertility. Mean age was 31.1 ± 4.8 years for group 1 and 37.5 ± 3.8 for group 2 ($P = 0.001$). TPR and PR-B expression was significantly decreased in the epithelial cells of infertile women compared with fertile controls. Specifically, TPR epithelial h-score was 2.2 ± 0.2 for group 1 vs. 1.5 ± 0.2 for group 2 ($P = 0.01$), while PR-B epithelial h-score was 1.6 ± 0.2 for group 1 vs. 1.0 ± 0.1 for group 2 ($P = 0.04$). No significant difference was observed in the epithelial expression of integrins avb3 ($P = 0.52$). Furthermore, no significant difference was observed regarding the expression of any biomarker in stromal cells. When adjusting for the cause of infertility, TPR and PRB epithelial expression were significantly decreased in sub-groups 2a ($P = 0.01$ and $P = 0.03$ respectively) and 2d ($P = 0.001$ and $P = 0.006$ respectively) compared with group1, while expression was comparable between sub-groups 2b, 2c and group 1.
Limitations, reasons for caution: This study reports only on protein profiling but not on mRNA levels as well. However, to our knowledge, this is the first report of endometrial expression levels of progesterone receptors in the various sub-groups of infertility, indicating significantly impaired expression in women with unexplained infertility and poor ovarian reserve.
Wider implications of the findings: The reduced expression of progesterone receptors especially in women with unexplained infertility may present a major aitiopathogenetic cause of infertility in such cases. Endometrial biopsy should be performed in all women that intend to perform IVF in order to evaluate endometrial receptivity.
Trial registration number: The present trial has been approved by Institutional Review Board and Ethical Committee of AUTH (A9352/25.7.2012)

P-355 Effects of low doses of mifepristone on the human embryo implantation process in a three-dimensional human endometrial *in vitro* co-culture system

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Study question: Could low doses of mifepristone during the receptive phase affect embryo implantation and endometrial receptivity markers?

Summary answer: A low dose mifepristone (0.5 μM) inhibited embryo implantation in the *in vitro* model; expression of endometrial receptivity markers were similarly affected by both doses.
What is known already: Mifepristone is a progesterone antagonist. It has been previously studied both *in vivo* and *in vitro* for its effect on endometrial receptivity. Also, it is considered a potential drug for contraception. It has been shown that a high dose of 200 mg administered on day LH+2 can inhibit implantation due to its effect on endometrium. We investigate here the effects of lower doses mifepristone administered during receptive phase on endometrial receptivity in an *in vitro* model mimicking *in vivo* conditions.
Study design, size, duration: Three-dimensional cell cultures were constructed from endometrial epithelial and stromal cells. Cultures were primed for five days with progesterone and estrogen to reach a state mimicking the receptive phase of the human endometrium. The cell cultures were divided into three groups and treated with: 0.5 μM mifepristone ($n = 8$) or 0.05 μM ($n = 10$) or vehicle as control ($n = 10$). The groups were compared for embryo implantation rate and expression of endometrial receptivity markers.
Participants/materials, setting, methods: Endometrial biopsies were collected from healthy, fertile women on day LH+4. Epithelial and stromal cells were isolated. Stromal cells were mixed with collagen gel and placed in a cell insert and epithelial cells seeded on top. After culturing five days, an embryo was added to each culture with either treatment/vehicle. Implantation rate was noted after five days and embryos removed. Cultures were processed for RNA extraction and real-time PCR analysis. *T*-test was used compare groups.
Main results and the role of chance: Implantation rate in the 0.5 μM mifepristone group was significantly ($p < 0.01$) reduced as 0/8 embryos attached to the endometrial construct, compared to 7/10 in the control group. Though implantation was also reduced in the 0.05 μM group (4/10 embryos attached) this was not significant. Interestingly, the expression of known endometrial receptivity markers FOXO1, FGF2, COUP-TFII, HOXA10, HAND2, OPN, LIF, PRL, PGR and IGFBP2 were expressed similarly in both treatment groups and differed from control. Furthermore, the expressions of IL6, IL1A and VEGFA were affected in the 0.05 μM group compared with the control. This indicates a dose dependent effect of mifepristone on the endometrium and that a low dose of 0.5 μM is sufficient to inhibit implantation as studied *in vitro*.

From the literature, it is known that 0.5 μM dose corresponds to an oral dose of 5 mg and we here show that exposure to mifepristone at this dose during the receptive phase *in vitro*, is enough to inhibit implantation.
Limitations, reasons for caution: The model used here is one of the best for endometrial receptivity and implantation, but it has limitations. It does not contain all cell types found *in vivo* and metabolic clearance of mifepristone is not the same as *in vivo*.
Wider implications of the findings: The results from this study take us a step closer towards rationalized use of mifepristone during receptive phase for preventing unwanted pregnancies.
Trial registration number: N/A.

P-356 *In vitro* evaluation of therapeutic effects of red ginseng and identification of associated miRNAs in endometriosis

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Study question: What is the potential therapeutic effects of red ginseng and the target miRNAs that are associated with therapeutic effects of red ginseng on endometriosis?
Summary answer: We identified that red ginseng induces alteration of the proliferative and fibrotic properties of the endometrial cells in endometriosis patients, and several miRNAs are involved.
What is known already: Recent discoveries indicate that several miRNAs are involved in the pathogenesis of endometriosis and red ginseng, especially Rg3-enriched extracts, is known to have anti-inflammatory and anti-proliferative effects in various cell types. We hypothesized that red ginseng may have similar effects in endometriosis through modulation of miRNAs.
Study design, size, duration: This study involved 20 cases of endometrial stromal cells from the patients with endometriosis, Ishikawa cell lines, and 10 endometrial cells from the patients without endometriosis (control group). All the cells were treated with Rg3-enriched red ginseng extracts and the test results were obtained after 48 h of red ginseng treatment.
Participants/materials, setting, methods: MTT assay was performed to determine the adequate red ginseng dose. Primary endometrial cells from the patients with endometriosis and Ishikawa cell lines were cultured and treated with red ginseng for 48 h. Real-time PCR was performed to analyze Ki-67, Col-1 and CTGF and microRNA microarray analysis was performed before and after red ginseng treatment. Contraction and migration assay was performed to evaluate fibrosis and migration potentials of the cells after red ginseng treatment.
Main results and the role of chance: The endometrial cells from the patients with endometriosis and Ishikawa cell lines were cultured and treated with 0.4 mg/ml of red ginseng extract for 48 h and the cell proliferation marker, Ki-67, and the fibrosis markers, Col-1 and CTGF (connective tissue growth factor), were significantly decreased in endometrial stromal cells (Ki-67; $p = 0.030$, Col-1; $p = 0.004$, CTGF; $p = 0.001$), and showed no significant changes except Col-1 ($p = 0.012$) in Ishikawa cell lines. MiRNA microarray revealed several miRNAs that were found to be differentially expressed after red ginseng treatments. Among those miRNAs, we identified mir-27b-5p, which has been implicated to be involved in modulating fibrotic responses from previous studies. The expressions of mir-27b-5p were significantly lower in the endometrium of patients with endometriosis compared to those without endometriosis ($p = 0.037$), and red ginseng treatment for 48 h significantly increased the expression of this miRNA ($p = 0.02$). Additionally, we also identified mir-18b-5p, which is known to target CTGF, and the expression of mir-18b-5p showed trends toward decreased expressions after red ginseng treatment. ($p = 0.063$). Contraction assay revealed significant reduction of contraction in endometrial cells from the patients with endometriosis after red ginseng treatment but no significant changes were noted in migration potentials of the treated cells.
Limitations, reasons for caution: This study was yet performed only *in vitro*. More data from *in vivo* model is needed for more conclusive results.
Wider implications of the findings: Red ginseng effectively alters fibrotic properties of endometrial stromal cells from the patients with endometriosis, and inhibition of fibrosis by red ginseng seems to be associated with modulation of miRNAs expressions. Red ginseng and modulation of associated miRNAs may provide new therapeutic approach for endometriosis.
Trial registration number: Not required.

P-357 Differential profile of transcripts detected by RNA-Seq in eutopic endometrium of infertile women with endometriosis and fertile controls during the implantation window

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Study question: Is the eutopic endometrium of infertile women with endometriosis molecularly different from the eutopic endometrium of fertile women during the implantation window?
Summary answer: The eutopic endometrium of infertile women with endometriosis appears to be molecularly similar to fertile women during the implantation window, without considering the disease stages.
What is known already: Some studies suggest that the expression of critical molecules for the implantation process may be altered in eutopic endometrium from infertile women with the disease. However, all of them have methodological limitations and none evaluated by RNA-Seq all transcripts differentially expressed in eutopic endometrium of infertile women with endometriosis during the implantation window. This information would allow a more complete understanding of this event in these patients and contribute to the elucidation of the mechanisms involved in the etiopathogenesis of disease-related infertility.
Study design, size, duration: We performed a prospective case-control study from November 2011 to November 2015 at the Human Reproduction Division

of the University Hospital HCFMRP-USP. The sample size was calculated to identify or rule out a major difference between the groups, so that 5 patients each would be sufficient to rule out a two standard deviation difference with a power of 90% and alpha of 5%.

Participants/materials, setting, methods: Endometrial biopsies were collected during the implantation window (confirmed by histological dating, according to Noyes criteria) from 6 infertile patients with endometriosis (3 endometriosis I/II, 3 endometriosis III/IV), 6 infertile controls and 5 fertile controls. The RNA-Seq was performed using the Illumina platform HISEQ2500, High Output, with paired-end library. The normalization of the data and the analysis of differential expression were performed using the R statistical environment DESeq2 package.

Main results and the role of chance: We did not identify any differentially expressed transcripts between the infertile and fertile control groups and between endometriosis (with no regard to the stage of the disease) and fertile control groups. However, five differentially expressed genes (*SCUBE1, CCL20, LGALS9C, TRIM29* and *WNT11*) were identified in the group with endometriosis III/IV, and 1 (*KANSL1-AS1*) in the endometriosis I/II group compared to fertile controls. Two differentially expressed genes (*KANSL1-AS1* and *VGLL3*) were identified by comparing the endometriosis I/II and the endometriosis III/IV groups. According to the enrichment analysis, although the differentially expressed genes identified in the eutopic endometrium of infertile patients with advanced disease do not belong to the same pathway, most of them are involved in the biological processes of cell proliferation, vascularization, immune and inflammatory response, cell fate and chemokine and cytokines signaling.

Limitations, reasons for caution: The main limitation was the small sample size evaluated due to the restrictive eligibility criteria adopted, limiting the generalizability of the results obtained. On the other hand, the strict eligibility criteria eliminated factors potentially related to the impairment of endometrial receptivity, increasing the internal validity of the study.

Wider implications of the findings: We evidenced no differentially expressed transcripts comparing the eutopic endometrium of infertile women with endometriosis and fertile controls. The subdivision of endometriosis group into disease stages suggests molecular differences, especially in advanced disease. Future studies with appropriate size are necessary to investigate these deregulated genes in the different endometriosis stages.

Trial registration number: This is not a clinical trial.

P-358 Is luteal phase support successfully performed using 25 mg/day of subcutaneous progesterone in controlled ovarian stimulation cycles with GnRH agonist triggering?

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Study question: Is 25 mg/day subcutaneous progesterone as effective as 50 mg/day intramuscular progesterone for luteal phase support in controlled ovarian stimulated cycles with GnRH agonist triggering?

Summary answer: 25 mg/day subcutaneous progesterone is as effective as 50 mg/day intramuscular progesterone preparing the endometrium in controlled ovarian stimulated cycles with GnRH agonist triggering.

What is known already: Daily administration of 25 or 50 mg of Subcutaneous Progesterone (SCP) has shown the same predecidual transformation in endometrial biopsies. It also has been indicated that Luteal Phase Support (LPS) with 25 mg/day SCP vs. vaginal progesterone had non significant differences in IVF cycles clinical outcomes.

LPS is necessary to prepare the endometrium for embryo implantation, specially in controlled ovarian stimulated (COS) cycles where a GnRH agonist is used for triggering. For these cases 50 mg/day Intramuscular Progesterone (IMP) is often used in clinical practice to improve clinical outcome.

Study design, size, duration: After COS antagonist protocol with standard doses of subcutaneous FSH 150–225 IU/day, 24 donors were randomized in one of two groups of this eight months interventional and randomized clinical trial. Follicle puncture was done 36 h after a bolus of GnRH agonist. Age, BMI, endometrial thickness (>7 mm) and blood levels of Luteinizing Hormone, Estradiol, Progesterone on P-2, P0, and P+5 (according to oocyte recovery day) were evaluated. Endometrial histology and transcriptomics were analyzed.

Participants/materials, setting, methods: 24 oocyte donors were included in one of two groups after COS antagonist protocole with GnRH agonist (triptorelina) triggering. They received daily progesterone starting on oocyte recovery day (P0): 25 mg SCP Prolutex® (n = 12) or 50 mg IMP Prontogest® (n = 12). Endometrial biopsies were collected on day P+5 in 23 donors. Endometrial receptivity transcriptomics profile was measured by using Endometrial Receptivity customized Array (ERA), and histological endometrial dating was performed by two different pathologists.

Main results and the role of chance: Statistical description of both treatments showed homogeneous distributions between them regarding population variables and COS cycle parameters. Only progesterone blood levels at day P+5 was significantly different between groups (p-value = 0.0001). From the histological point of view, all the biopsies were dated in the secretory phase: 65.2% in media secretory, 13% in early secretory and 21.7% in late secretory phase. Endometrial histology showed non significant differences in the secretory dating phase between SCP and IMP (p-value = 0.3395).

Regarding deeper transcriptomic level, 88.24% of ERA genes showed the same trend of gene expression values in both treatments. Despite having the statistical power of 92% to detect differences in gene expression, it only was obtained a few changes in the global transcriptomic profile, 28 differential expressed genes have been detected, and four of them (1.6%) were changing significantly with a high gene expression difference between treatments (adj-p-value < 0.05, |FC| > 3), specially IGFBP1 (adj-p-value = 5.05E-04, Fold Change = 15.32) and CXCL13 (adj-p-value = 1.86E-06, Fold Change = 8.37) up and down regulated respectively in SCP.

Limitations, reasons for caution: Although histological or global molecular differences were not found in endometrial preparation of this donor population, further research is needed in IVF cycles in order to confirm that those few molecular changes are not affecting clinical pregnancy outcome.

Wider implications of the findings: Our findings, using histological and deeper transcriptomic endometrial evaluation, suggest that 25 mg/day of SCP in COS cycles with GnRH agonist triggering could be effective for LPS. With the same approach, LPS in IVF cycles could be improved with 25 mg/day of SCP for the wellness of patients.

Trial registration number: EudraCT Number: 2015-000290-12, ClinicalTrials.gov ID: NCT02567552.

P-359 GSTM1 polymorphisms in endometriosis in Greek women

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Study question: Can the absence of the *GSTM1(Glutathione S-transferase mu 1)* allele be associated with endometriosis?

Summary answer: Absence of this allele may be associated with endometriosis and tends to have a statistically significant association with the age of initiation of the symptoms.

What is known already: *GSTM1* is a member of the GST family proteins which catalyze the conjugation of glutathione to potentially genotoxic compounds. It is also shown that the homozygous null deletions of *GSTM1* cause the loss of its encoded enzyme, participating in the detoxification of a large range of environmental contaminants. Previous studies suggested that the null genotype of *GSTM1* can lead to high endometriosis risk, especially in European and Asian populations. Thus, it might promote the occurrence and development of endometriosis.

Study design, size, duration: We studied 49 Greek women with no miscarriages and with at least one successful pregnancy and 29 Greek women with endometriosis.

Participants/materials, setting, methods: The study was conducted in a University hospital. We used endometriotic tissue from 29 women with infertility and histologically confirmed endometriosis, as well as blood samples from 49 controls, who have had at least one successful pregnancy. For each sample, genomic DNA was isolated and PCR was performed using specific for the *GSTM1* gene primers. The presence of the *GSTM1* allele was identified by gel electrophoresis.

Main results and the role of chance: According to statistical analysis, 34,5% of endometriosis cases have the homozygous null genotype in contrast with 18,4% of controls (95% CI, p = 0,171). The absence of allele tends to have a statistically significant association with the age of initiation of symptoms (p = 0.044) Additionally when blood sample and endometriotic tissue from the same woman were studied for the presence of the allele, a different genotype was found in 10 out of 29 women. To our knowledge this is the first study in which this polymorphism is detected in different tissues. The above findings may be relevant to a cellular and molecular explanation of the causes of endometriosis.
Limitations, reasons for caution: This is a prospective study. The presence of GSTM1 polymorphism will be studied in more samples to verify the results.
Wider implications of the findings: Concerning the mechanisms underlying endometriosis a possible impact of environmental factors may be evaluated. The establishment of a genetic profile may improve the prediction of the reappearance of endometriotic lesions. An association of the polymorphism with clinical characteristics may help clinicians in consulting and planning treatment in patients with endometriosis.
Trial registration number: Not a trial.

P-360 Effect of endometrial injury on reproductive outcome in assisted reproduction: an observational study

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Study question: To analyse the effect of endometrial injury before IVF or ICSI on clinical pregnancy outcome.
Summary answer: Endometrial scratching performed before IVF/ICSI increased clinical pregnancy outcome in the second attempt of IVF/ICSI, unlike overall comparison.
What is known already: Since over 10 years, endometrial scratching is performed prior to IVF, hoping for better pregnancy outcome. However, there are no large randomised studies which support the routine clinical practice. Studies have shown to increase pregnancy rate by 10–70% in patients who had endometrial injury compared to women who didn't have. The endometrial injury could enhance chances of implantation, by modulating implanting gene expressions which help by increasing production of cytokines, interleukins, growth factors, macrophages, dendritic cells and helping in decidualisation.
Study design, size, duration: It is retrospective observational study at centre for reproductive medicine at the University Hospital. All women who had IVF/ICSI including embryo tranfer between January 2015 and July 2015 were included. There were 106 patients who had endometrial scratch (informed choice) and 315 patients who did not have the endometrial scratch. Demographic data and reproductive outcome were compared between the two groups using the chi-square test.
Participants/materials, setting, methods: The patient's information from the data base was recorded. Women who had the endometrial scratch, the duration between endometrial scratch and oocytes retrieval were recorded. All patients had endometrial scratch by pipelle. The sample obtained was discarded. 1 or 2 embryos were transferred on day 2, 3 or 5 after eggs retrieval. The luteal phase was supported by progesterone pessary and injections in some. The pregnancy test was done 14 days after transfer.
Main results and the role of chance: Out of 421 patients who under went IVF/ICSI, 106 patients had endometrial scratch. The baseline data were similar in both the groups and statistically not significant. There was no difference between age, parity, number of attempts of IVF/ICSI, and treatment protocol(long protocol or antagonist cycle) in 2 groups. 61/106 (57%) of patients who had endometrial scratch got pregnant compared to 159/315 (51%) percent in control group(p value 0.20, statistically not significant). Patients who had second attempt of IVF/ICSI in endometrial scratch group, 22/29 (75%) got pregnant.
Limitations, reasons for caution: It is a retrospective observational study. The number of patients was less, hence, it is difficult to suggest which patients would benefit. All the patients did not have intervention in the previous proliferative phase of menstrual cycle as suggested by other studies.
Wider implications of the findings: The study suggests endometrial injury in the second attempt of IVF/ICSI increases pregnancy rate. Previous studies have shown endometrial scratch helps to improve pregnancy rate in women with unexplained recurrent embryo implantation failure.
Trial registration number: –

P-361 Transvaginal ultrasonography accelerated video recording is a reliable method for the assessment of uterine peristalsis before embryo transfer

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Study question: It is accelerated video recording a useful tool to measure uterine contractions?
Summary answer: Accelerated video recording of transvaginal ultrasonography scan is a reliable method to assess the uterine contractions before embryo transfer in egg recipients with substitutive cycle.
What is known already: Implantation rate after embryo transfer is a limiting factor in the success of IVF treatments, thus efforts must be devoted to improving uterine receptivity in clinical practice. Uterine peristaltic wave frequency before embryo transfer has been inversely related to the clinical pregnancy and treatment with oxytocin antagonists has been suggested to improve the outcomes of IVF treatments. In the near future, the assessment of uterine contractions could be a useful tool in the clinical practice.
Study design, size, duration: Study to evaluate the variability intra- and inter-observer. Uterine peristaltic activity was prospectively assessed before blastocyst transfer in 20 oocyte recipients between March and December 2015.
Participants/materials, setting, methods: 2D scan of mid-sagittal plane of the uterus was recorded while a 6 min video. The records were analysed at 20× regular speed using a VLC media player. Three independent experienced observers performed three evaluations of every video. Intraclass correlation coefficient (ICC) was used to validate the variability.
Main results and the role of chance: All patients presented contractions during the evaluation. The average was 1.64 per min (1.5–1.74). The ICC to evaluate the inter-observer variability was 0.839 (0.7 a 0.93; p = 0.000). Regarding the intra-observer variability, the ICC was 0.95 (0.9 a 0.98; p = 0.000) for the observer "A", 0.948 (0.9 a 0.98; p = 0.000) for the observer "B" and 0.839 (0.7 a 0.93; p = 0.000) for the observer "C".
Limitations, reasons for caution: All the participants in the study were egg recipients and results could be different in embryo transfers after ovarian stimulation.
Wider implications of the findings: Having this reliable tool, efforts must be carried out to clarify whether the uterine peristalsis may be a marker of uterine receptivity. In this case, trials may be developed to investigate the possibility to reduce contractility.
Trial registration number: Nothing to disclose.

P-362 Intralipid in women with recurrent implantation failure in IVF/ICSI cycles. a double blinded randomised controlled trial

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Study question: Does Intralipid use in women with recurrent implantation failure in IVF/ICSI cycles improve Live birth rates?
Summary answer: Intralipid use in women with Recurrent Implantation Failure in IVF/ICSI cycles is not associated with improved Live birth rates.
What is known already: Recurrent embryo implantation failure (RIF) is a disorder with potentially devastating physiological and psychological manifestations for those affected. Although its prevalence is not uncommon, many of the mechanisms involved still require elucidation. Evidence from both animal and human studies suggests that Intralipid, administered intravenously, may enhance suppression of natural killer cell activity. Up to our knowledge, this is the first RCT assessing the efficacy and safety of Intralipid in women with recurrent implantation failure after IVF/ICSI cycles.

Study design, size, duration: A Double Blinded Randomised Controlled Trial was performed from October 2012 till April 2015 on 100 women with history of Recurrent Implantation Failure after IVF/ICSI cycles.
Participants/materials, setting, methods: 100 Women with recurrent implantation failure were randomised into two groups.
Group I: Subjects (n = 50) who were treated with Intralipid 20% starting 6–7 days before embryo transfer followed by a repeated dose in case of a positive pregnancy test. ***Group II:*** Subjects (n = 50) who were treated with placebo starting 6–7 days before embryo transfer followed by a repeated dose in case of a positive pregnancy test. Clinical pregnancy rates and live birth rates were compared in both groups.
Main results and the role of chance: There was no evidence of improved live birth and pregnancy rates in women who received Intralipid Infusion compared to placebo. Results were reported according to intention to treat analysis ((ITT) with a relative risk (RR) of 1.56, 95% CI [0.74,3.27], P value 0.244 for live birth rates and RR 1.46, 95% CI [0.81,2.64], P value 0.207 for clinical pregnancy rates. 2 live birth cases in the Intralipid arm were born with middle ear anomaly that required corrective surgery.
Limitations, reasons for caution: There was a tendency towards increase in pregnancy and live birth rates in the intralipid arm, however this increase was not statistically significant. This may be due to small sample size. Also, finding of middle ear congenital anomalies may be related to repeated intralipid dosing practised in our IVF centre..
Wider implications of the findings: According to our findings, intralipid is not only not effective in improving live birth and pregnancy rates, It is proven to be associated with congenital middle ear anomalies.
Trial registration number: Trial registration number, Clinical Trials. gov. ID NCT02487940.

POSTER VIEWING SESSION

ETHICS AND LAW

P-363 What are the appropriate roles of induced germ cells at fertility clinics?

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Study question: In due consideration of the advances in stem cell research, what purpose justifies the clinical use of germ cells derived from human pluripotent stem cells?
Summary answer: Autologous induced pluripotent stem cell (iPSC)-derived spermatozoa might become a reproductive option for male cancer survivors.
What is known already: Without using donor gametes, there is no assisted reproductive technology (ART) treatment for patients with few or no gametes. Recent research suggests that germ cells may be induced from a patient's somatic cells by controlling the cell fate, which may be integrated into new ART treatments for infertile couples and even same-sex couples in the future. There are currently no clinically validated induced germ cells. However, with the advent of genome editing, stem cell research will advance rapidly, urging us to consider the potential upcoming ethical and societal issues associated with the clinical use of induced germ cells.
Study design, size, duration: The risks, burdens and limitations of three approaches for inducing human germ cells from pluripotent stem cells were scrutinized. Since the benefits of induced germ cells are likely found in self-use by a couple to have a genetically related child, the clinical justification was contemplated regarding four scenarios in same sex couples, in couples having a sick child and in patients of older and younger ages. Finally, the relevant regulatory issues were considered.
Participants/materials, setting, methods: This analysis includes works published in English until August 2015, and which were available through PubMed.
Main results and the role of chance: Germ cell induction involves the reversal of human development, thus underscoring the need for ensuring the developmental potential of induced germ cells and for assuring the health of the resulting offspring. Two induction approaches using autologous iPSCs deserve further consideration, despite the possibility of genomic instability during reprogramming as well as the cumulative burden of *de novo* mutations in the somatic cells of origin. The induction of germ cells from cloned embryonic stem cells was rejected for genetic and ethical reasons. Taking into account the fact that germ cell induction is currently far more advanced in male cells than in female cells, the ethical analysis of the four scenarios concluded that the reproductive use of induced spermatozoa might be justifiable as an option for males who have lost viable gametes due to cancer treatment. The first reproductive use of induced spermatozoa should occur in a clinical trial. In the provision of informed consent, the predictable risks, such as embryonic arrest, implantation failure, miscarriage and the birth of a child with congenital anomalies, must be carefully explained.
Limitations, reasons for caution: The study of human embryos alone is insufficient for avoiding the risks associated with ART treatment. Although the intergenerational monitoring of mouse offspring derived from induced germ cells will provide valuable insight into safety, additional studies using other animal species, particularly non-human primates, are needed before contemplating clinical applications.
Wider implications of the findings: Further social discussions about the appropriate role of ART using induced germ cells should be held before initiating this new ART. A legal analysis demanded the establishment of appropriate regulations in order to avoid troubles associated with the misuse of induced germ cells at fertility clinics.
Trial registration number: None.

P-364 Assessing the use of assisted reproductive technology in the United States by non-U.S. residents

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Study question: To assess the frequency and trends of assisted reproductive technology (ART) use in the United States by non-residents (country of residence, treatments received, and outcomes).
Summary answer: ART use among non-residents is increasing in the U.S. and involves higher use of oocyte donation, gestational carriers, and pre-implantation genetic diagnosis than among residents.
What is known already: Travel across national borders in pursuit of treatment with ART raises medical and public health concerns. Little is known about the number of patients participating in cross-border reproductive care (CBRC), the specific ART treatments these patients receive, or the outcomes of these treatments.
Study design, size, duration: We conducted a population-based retrospective cohort study of all ART cycles (n = 1,080,935) reported to the National ART Surveillance System (NASS) in the U.S. from January 2006 to December 2012.
Participants/materials, setting, methods: We used NASS data to assess the frequency and trends of ART cycles performed by non-residents in the U.S.; to assess whether non-resident patients disproportionately used donated oocytes, gestational carriers, and pre-implantation genetic diagnosis; and to compare embryo transfer rates, live birth rates, and multiple birth rates for U.S. resident and non-resident ART cycles.
Main results and the role of chance: The non-resident share of all U.S. ART cycles grew each year, increasing from 1.2% (n = 1,683) in 2006 to 2.2% (n = 3,966) in 2012 and, during that period, patients from 141 countries had ART treatment in the U.S. The data also indicate that, compared with resident ART cycles, non-resident cycles had higher use of oocyte donation (10.7% vs. 41.8%), gestational carriers (1.6% vs. 12.2%), and PGD (5.1% vs. 18.1%). U.S. resident and non-resident cycles had similar average number of embryos transferred, live birth and multiple birth rates.
Limitations, reasons for caution: Although NASS collects information on residency status for all individuals using ART in the U.S., misclassification is possible. In addition, NASS lacks information on the reasons that non-residents travel to the U.S. for ART treatment.

Wider implications of the findings: CBRC makes up a small but growing share of ART usage in the U.S. The disproportionate use by non-resident CBRC patients of third-party ART techniques, involving oocyte donation, gestational carriers, and pre-implantation genetic diagnosis raises important ethical, policy and legal issues of international scope.
Trial registration number: Not applicable.

P-365 Attitudes of altruistic anonymous and identity-release oocyte donors towards future contact with donor offspring

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Study question: What are oocyte donors' attitudes towards future contact with donor offspring, will they share information about the donation(s) with children of their own?
Summary answer: Of past donors, 90% felt pleased or had neutral feelings towards contact with donor offspring and 91% had told/planned to tell their own children.
What is known already: There is limited knowledge about past donors´ attitudes about being contacted by donor offspring and feelings towards the potential donor offspring. One study of women who had donated in 2007-09 in an egg-sharing program in London found that 27 donors expressed goodwill and curiosity towards potential children while 3 were negative or ambivalent. Among identity-release donors a subset of donors has also been reported to have negative attitudes towards contact with donor offspring. No study has reported how donors will react to contact between donor offspring and their own children.
Study design, size, duration: A retrospective cross-sectional survey of all women who had donated oocytes between 1990 and 2012 at three fertility clinics in Finland was carried out in spring 2013. A self-administered questionnaire was sent out to a total of 569 former oocyte donors including anonymous or identity-release donors unknown or known to the recipient couple.
Participants/materials, setting, methods: The response rate was 75.3%. 371 donors were unknown to the recipient and were included in the study. The mean follow-up time after the donation was 11.4 years. Before 2008, donors were officially non-identifiable (anonymous) but they had a possibility to report to the clinic willingness to be contacted by the donor offspring. After 2008 all persons born as a result of gamete donation can, from the age of 18, receive identifying donor information (identity-release).
Main results and the role of chance: To learn whether the attitudes of donors was affected by the Finnish Assisted Reproduction Technology (ART) Act of 2007 we divided the 371 respondents into two groups: Group 1: women whose first donation took place between 1990 and 2007 ($n = 290$; voluntary registration 61.3%) and Group 2: women whose first donation took place between 2008 and 2012 ($n = 68$). Forty-one percent thought that the parents should inform the child of the way of conception (Group 1 36% vs. Group 2 64%; $p < 0.001$). Of the donors, 72.4% would be pleased about being contacted by donor offspring, 20.3% felt neutral and 7.4% expressed negative feelings towards contact with a donor offspring (no significant group differences).

Among donors who had children of their own ($n = 308$; 83%), 48% had informed and 43% planned to inform their own children, whereas 9% had decided not to inform. Disclosure about donation to own children did not differ between the groups. The majority (69%) of donors with children of their own would support their children if they would want to maintain contact with donor offspring. Less than one percent was against or would be upset about contact between donor offspring and donor's own children.
Limitations, reasons for caution: Although the response rate was high, 25% of all former donors in the three participating clinics could not be included due to lack of response.
Wider implications of the findings: Through 22 years and independently of the 2007 ART legislation, the majority of past donors have positive or neutral feelings towards being contacted by a person born of the donation. Based on these results legislation that promotes use of identifiable donors can be recommended.
Trial registration number: None.

P-366 The role of ethical consultation services in private IVF centers in the United States

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Study question: What is the optimal role and composition for ethics committees in private IVF centers in the United States?
Summary answer: The optimal role and composition of private IVF center ethics committees is not yet known. We describe the role we have found for expert consultation.
What is known already: Much has been written about ethics committees in large hospitals; however, little is written about the role, composition and organization of private ethics committees. Private IVF centers have, at best, very limited access to hospital or university based ethics committees and are potentially left adrift without ready access to expert ethical advice. As technology in assisted reproduction advances we are faced increasingly with difficult requests to perform procedures which challenge established ethical principles and social morays.
Study design, size, duration: None.
Participants/materials, setting, methods: Large academic private IVF center. University based Bioethics Center.
Main results and the role of chance: Herein we report the evolution of our experience from an informal internal ethics committee to formal external expert consultation. Initially, cases with ethical quandaries were brought to our quality assurance committee, comprised of physicians, nurses, embryologists and mental health professionals.

Case discussions focused on perceived ethical principles. We came to a general consensus regarding offering or denying treatment by treating each case as a unique event without subjecting the points of contention to a systematic application of the Belmont principles.

On occasions where consensus could not be reached, we sought a formal opinion from bioethicists at our hospital of record.

Faced with an increasing number of issues as technology evolved, we contracted with Harvard Medical School's Center for Bioethics. Under this unique agreement, we will develop clinical guidelines and protocols regarding ethically charged scenarios to help guide decision making in a thoughtful, formal and structured manner through application of ethical principles.

Our ethics consultation team consists of a multi-disciplinary group invited from a wide variety of specialties and backgrounds. Further, the team has developed a curriculum which involves the teaching of ethical principles to our staff and fellows through a combination of didactic as well as case based discussions.
Limitations, reasons for caution: A formal ethics consultation service may not be the solution for every IVF clinic. However, our hope is to draw upon our experience in this collaboration and create a casebook as a guide for smaller practices.
Wider implications of the findings: Assisted Reproduction is fraught with ethical questions. Application of ethical principles in a meaningful way requires specific expertise. Incorporation of rigorous ethical analysis to the practice of assisted reproduction may be facilitated by the creation of a casebook as further guidance to providers.
Trial registration number: None.

P-367 The general public's opinion on stem cell based fertility treatments: who should decide and on what?

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Study question: Should individual patients and their physicians or a national committee of representatives decide on the conditions for accepting stem cell based fertility treatments?

Summary answer: According to the Dutch general public, patients and physicians cannot decide themselves on the conditions for accepting stem cell based fertility treatments.
What is known already: Current fertility treatments do not result in parenthood for one in three couples. Several stem cell based fertility treatments are being developed for these couples. This spurs a debate on the legitimacy of treatment indications and on the conditions for clinical introduction. Whether patients and their physicians can decide themselves on the conditions for accepting these treatments or whether this should be decided on a national level is contested. In line with our democratic principles and as they include and/or represent the targeted couples and the future children, we asked the opinion of the general public.
Study design, size, duration: In November 2015 a cross-sectional survey was disseminated among 1250 respondents of an actively recruited panel that is representative of the general Dutch adult population in terms of several demographic characteristics.
Participants/materials, setting, methods: Conditions important for accepting stem cell based fertility treatments were defined by literature review and interviews with patients and gynecologists. A non-directive questionnaire was developed, reviewed by experts from seven different disciplines (e.g., political science) and tested among the general public. Finally, we asked whether the acceptability of nine conditions should be defined by patients and their physicians or by a national committee of representatives. Regression analysis examined whether demographic characteristics were determining.
Main results and the role of chance: A total of 778 members of the general public completed the survey (response rate: 62%). Fifteen percent of them had sought medical assistance for infertility. Most respondents (51%) indicated the decision-maker should differ per condition, instead of all conditions being decided on by a national committee of representatives (35%) or individual patients and their physicians (14%). The vast majority (≥75%) stated that a national committee of representatives should decide on the acceptability of the potential risks of major birth defects and of late-onset chronic diseases in the child. According to most respondents the same applied to: the destruction of embryos (72%), the risk of minor birth defects (68%), the risk of cancer for the intended parents (66%), the limited similarities between the treatment and natural conception (55%), and the limited success rates (53%). Individual patients and their physicians could solely decide on high treatment costs (53%) and high treatment burden (55%). Depending on the condition, the national committee of representatives should be an independent medical-ethical board (29–50%), a professional association of physicians (11–17%) or the government (11–16%). Respondents who highly valued religion or had conservative political views were more likely to prefer a national committee of representatives.
Limitations, reasons for caution: The perspective of the general public may change over time and might differ across countries as it is influenced by many factors. The likelihood at which harmful consequences will occur in humans is and will remain uncertain prior to large-scale clinical application.
Wider implications of the findings: A responsible and well-designed decision-making process should be put into place for deciding on the required conditions for clinical application of stem cell based fertility treatments, rather than waiting for patients and physicians to take this decision. The general public supports an independent medical-ethical board as the most prominent decision-maker.
Trial registration number: Not applicable.

POSTER VIEWING SESSION

FEMALE (IN)FERTILITY

P-368 Cost-effectiveness of social freezing in Germany – estimates based on a Markov model

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Study question: Is "social freezing" in Germany cost effective if compared to natural conception and IVF?
Summary answer: Based on actual pregnancy probabilities and pricing social freezing is not cost effective today.
What is known already: As the age of women bearing their first child is increasing in today's society and fertility declines with increasing female age, social freezing is an emerging procedure that offers the possibility to push childbearing age at wish. For oocyte freezing healthy women need to endure medical procedures with extra risk and cost. As in Germany cost is not reimbursed, it is of upmost interest to debate cost efficiency of this technique.

We therefore developed a Markov model to estimate cost-effectiveness of different strategies to use oocyte freezing to postpone pregnancy for social indication based on the numbers given in Germany.
Study design, size, duration: We developed three strategies to delay childbearing until the age of 40. Reference strategy: no action until 40 and if no spontaneous conception (SC) occurs after one year, three cycles of standard IVF. Strategy I: three cycles of oocyte freezing at the age of 25/28/30/35/38 and thawing at 41 after SC for one year and strategy II: no action until the age of 40, followed by trying SC.
Participants/materials, setting, methods: We used an adapted Markov model to estimate cost-effectiveness between the three Strategies. Chances of a pregnancy were calculated with a life birth after standard IVF/ICSI, after IVF with thawed oocytes and after natural conception. The cumulative life birth rate to conceive within 5 years, cost per woman, cost per life birth were calculated. The probabilities to move from one state into another and the cost were based on published data (e.g., German-IVF-Registry (DIR)).
Main results and the role of chance: Cumulative life birth rate at 45, after five years trying to conceive for the reference strategy, NC and IVF/ICSI at the age of 41 was 73.03%, cost per women were 7,790.22€ and per life birth 10,666.89€. Cumulative live birth rate for strategy I, oocyte freezing at the age of 25/28/30/35/38 using them at the age of 41 were in the range from 87.00% (25) to 75.46%(38). Cost per woman from 9,880.05€ (25) to 13,928.10€ (38) and cost per life birth 11,346.57€ (25) to 18,457.17€ (38). Cumulative life birth rate for strategy II, SC without any treatment was 70.29%. For women using strategy I the chance for a life birth increases by 3.33% (38) to 19.12% (25) compared to IVF/ICSI and by 7.35% (38) to 23.76% (25) compared to natural conception. Per 100 women per strategy, this would result in 2 (38) to 14 (25) additional life births with social freezing compared to IVF/ICSI and 5 (38) to 17 (25) compared to natural conception. Cost increases by 26.83% (25) to 78.79% (38) for social freezing compared to IVF/ICSI. If we compare IVF/ICSI to natural conception, the chances increase by 3%. That would be 4 additional life births.
Limitations, reasons for caution: This study has several limitations. Even if effect of age on fertility has been widely studied data on natural conception especially at higher age are limited. The same is true for data on oocyte freezing, fertilization and pregnancy rates. The model might therefore vary for different assumptions.
Wider implications of the findings: Based on our results social freezing in Germany leads to additional pregnancies but also to higher cost today. This should be taken into account when counseling our patients and should be re-evaluated in the future with adapted numbers, e.g., upcoming data on success rates.
Trial registration number: N/A.

P-369 Pain perception and side effects during sonohysterography with balloon catheters: a randomized comparative study of cervical with uterine catheter placement

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Study question: To assess whether the location of the balloon catheter either the uterus or cervix during sonohysterography can affect the pain perception and vasovagal reactions rate.
Summary answer: The position of the catheter did affect the magnitude of pain and vasovagal reaction rates between the groups.

What is known already: Saline infusion sonohysterography and other intrauterine investigations methods may cause uterine cramping, pain and vasovagal reactions. According to the few previous studies the position of catheter placement can affect the magnitude of pain and amount of saline required during the sonohysterography.
Study design, size, duration: This study was a double blind randomized controlled trial on 300 patients undergoing sonohysterography between May 2012 and May 2014. Eligible women were randomly allocated to undergo intrauterine or intracervical balloon catheter placement. The Primary outcome measures included the degrees of pain perceptions after inflation and deflation of balloon catheter at the end of sonohysterography. Secondary outcomes included total time of procedure, total volume of required saline, spontaneous catheter expulsion and vasovagal reaction rates.
Participants/materials, setting, methods: The infertile patients aged 18–45 years were enrolled in this study. The examination was scheduled in the early follicular phase. A 2 lumen 6-French catheter was placed in the lower uterine segment or 1 cm into cervical canal and inflated with 1.2 mL of sterile saline. Pain was measured with a 10 point-VAS. Patients were monitored during and 30 min after the examination to diagnose and treat of intolerable pain and vasovagal reactions.
Main results and the role of chance: One hundred and forty eight subjects were randomly assigned to intracervical balloon insertion and 152 to intrauterine placement. There were no significant differences in inflation and deflation pain and total procedure time between two groups. Intracervical placement requires less significant volume of saline compared with intrauterine placement which reduces the risk of intrauterine infection and spread of malignant endometrial cells into the peritoneal cavity. The nulliparous women experienced significantly more pain after initial inflation ($P = 0.04$). Pain scores were not associated with patient's age, volume of saline infused and presence of pathology. But pain scores were significantly correlated with total procedure time.

Patients using intracervical balloon placement had a significantly higher rate of spontaneous expulsion of the catheter ($P = 0.004$). There were no statistically significant differences in occurrence of vasovagal reactions between two groups. None patient in both group experienced severe vasovagal reaction (manifesting as vomiting and syncope).
Limitations, reasons for caution: Due to slipping of the balloon from intracervical to intrauterine position and vice versa (about 10% of patients) outcome measures were assessed based on the intent-to-treat principle.
Wider implications of the findings: According our results, there is not sufficient evidence to suggest routine intracervical catheter placement for sonohysterography.

Intrauterine catheter placement can be considered in parous patients to avoid fluid reflux and spontaneous catheter expulsion. While intracervical catheter placement is suggested in nuliparous to improve patient satisfaction and to minimize perceived pain.
Trial registration number: NCT01936116.

P-370 A retrospective analysis of outcomes of 585 selectively reduced multiple pregnancies in IVF/ICSI-ET cycles

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Study question: To investigate whether selective fetal reduction in early pregnancy has any effects on obstetric and fetal outcomes of reduced multiple pregnancies produced through IVF/ICSI-ET cycles.
Summary answer: As compensating for multiple pregnancies, fetal reduction was effective in reducing the rate of multiple pregnancy, while it was not helpful for the pregnant outcome.
What is known already: As compared with primary singleton pregnancies, whether fetal reduction to singleton further compromises the outcomes of the reduced pregnancies has not reached an agreement. There were studies showing a comparable miscarriage rate, mean gestational age and neonatal weight when reduced singleton, twin, and triplet pregnancies compared to non-reduced pregnancies. While other studies found the rates of low birth weight and preeclampsia in reduced singleton were increased compared to the primary singleton, and the rates of preterm delivery and low birth weight of reduced twins were increased compared to the primary twins.
Study design, size, duration: A total of 1785 IVF/ICSI-ET cycles which reached clinical pregnancy at our IVF center from January 2002 to June 2014 were studied, including 585 selectively reduced multiple pregnancies and 1,200 primary singleton pregnancies which were selected by nested case control study method. Maternal and neonatal outcomes were compared between groups, with a value of $P < 0.05$ used for significance.
Participants/materials, setting, methods: Total of 585 cycles with fetal reduction to singleton were selected as study group, while 1,200 primary singleton cycles were set as control. The exclusion criteria included: (1) age >37, chromosomal abnormalities, uterine factors (fibroid, adenomyosis, deformity) and endocrine disorders (PCOS, thyroid disease and diabetes, etc.); (2) time of IVF/ICSI cycles ≥3; (3) intrauterine merger ectopic pregnancy and ectopic pregnancy.
Main results and the role of chance: The mean maternal age of the reduced singleton group was 30.1 ± 3.4, with no significant difference compared to the primary singleton group (30.3 ± 3.4). Both BMI and duration of infertility had no significant difference between the two groups. Comparing to primary singleton group, the number of embryos transferred, the incidence rates of pregnancy complications and preterm delivery were significantly higher ($P < 0.05$), while the complete abortion rate and gestational age were significantly lower ($P < 0.05$) in the reduced singleton group. There was also no significant difference in complications during delivery between the two groups.

The incidence rate of low birth weight, perinatal mortality and birth defects in the reduced singleton group was significantly ($P < 0.05$) higher than the primary singleton group. There was no significant difference in neonatal diseases between the two groups.
Limitations, reasons for caution: There are some limitations for our study, the most significant of which come from its retrospective nature. Besides, the lack of comparison in embryo quality between the two groups which might be the reason for early abortion. Further large and multicenter study will be needed for more solid conclusions.
Wider implications of the findings: Reduction surgery is only taken as a compensated measure after multiple pregnancies occurred, whereas to limit the number of transferred embryos is the essential way to improve the outcomes of IVF/ICSI-ET cycles. Moreover, prenatal follow-up should be reinforced after reduction to decrease risks during pregnancy.
Trial registration number: No trial registration number.

P-371 Body mass index and pregnancy outcomes after single blastocyst transfer: an evaluation of obese and morbidly obese (BMI >40 kg/m^2) North American women

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Study question: To examine the live birth rate (LBR) after single, "ideal-quality" blastocyst transfer (SBT), in morbidly obese patients from a North American population.
Summary answer: LBR after SBT decreases substantially above a maternal BMI of 35 kg/m^2, with a further decrease above a BMI of 45 kg/m^2.
What is known already: Maternal BMI has been shown to have a negative effect on pregnancy outcomes achieved with IVF. However, this effect can be partially overcome by the transfer of multiple embryos. Recent studies from European populations have shown poorer pregnancy outcomes after SBT in obese women (BMI 30–35 kg/m^2) compared to women of normal weight (BMI 20–25 kg/m^2). Yet, the effect of BMI on reproductive outcomes after SBT, has never been evaluated in women with a BMI above 35 kg/m^2 or in North American women. This is significant given the higher prevalence of morbid obesity in North America compared to European populations.
Study design, size, duration: This is a retrospective, cohort study including 520 nulliparous and multiparous women, less than 40 years of age, undergoing their first, "ideal-quality" SBT between August 2010 and March 2014 at a University Centre. Each patient was included only once. 88 women had a BMI of at least 30 kg/m^2. The maximum BMI in the study was 57 kg/m^2. Live birth was defined as an infant born with signs of life at >24 weeks GA.

Participants/materials, setting, methods: Exclusion criteria: congenital or acquired uterine anomalies, hydrosalpinges, or donor embryos. Study outcomes were reported based on BMI categories. Statistics were analyzed with logistic regression to control for confounding effects and multiplicity. The following confounders were controlled for: female age, previous pregnancies, previous term deliveries, BMI, smoking status, baseline FSH level and antral follicle count, duration of infertility, total gonadotropin dose, and stimulation protocol. Data is presented as mean ± standard deviation or percentage.
Main results and the role of chance: The mean age was 32.9 ± 3.4 years (range 22–39 years). The mean BMI was 24.8 ± 6.2 kg/m^2 (range 17.0–57.0 kg/m^2). Among female age, previous pregnancies, previous term deliveries, BMI, smoking status, baseline FSH level and antral follicle count, duration of infertility, total gonadotropin dose, and stimulation protocol, BMI was the only statistically significant predictor of LBR ($p = 0.02$), with the exception of a previous term delivery of a live infant ($P = 0.0001$). Pregnancy outcomes were reported by BMI categories, including: <20, 20–24.9, 25.0–29.9, 30–40, >40 kg/m^2. The pregnancy rate (PR) for each group was 45%, 62%, 45%, 36%, and 20%, respectively ($p = 0.004$); the clinical pregnancy rate (CPR) per group was 36%, 52%, 38%, 26%, and 10% ($p = 0.009$); and the LBR per group was 35%, 50%, 38%, 26% and 10% ($p = 0.03$). The CPR and LBR in morbidly, obese women (BMI >40 kg/m^2) was over 50% less than that of obese women (BMI 30–39.9 kg/m^2). Similarly, the LBR of normal weight women (BMI 20–24.9 kg/m^2) was five times that of morbidly, obese women (BMI >40 kg/m^2). BMI was a significant predictor of LBR even when excluding women with PCOS ($n = 373$; $p = 0.014$).
Limitations, reasons for caution: This study is limited by its retrospective nature. Further, randomized prospective trials are needed to validate the relationship between BMI and pregnancy outcomes following SBT in morbidly, obese women; especially since the transfer of multiple embryos results in poorer pregnancy outcomes as a function of BMI.
Wider implications of the findings: A BMI between 20 and 24.9 kg/m^2 is ideal for patients undergoing SBT. Pregnancy outcomes are worse in patients with a BMI <20 kg/m^2 or >35 kg/m^2, with a further drop above a BMI of 45 kg/m^2. This is the first study to examine the effects of morbid obesity after SBT.
Trial registration number: Not applicable.

P-372 Randomized, open trial comparing a modified double-lumen needle follicular flushing system with a single-lumen aspiration needle in IVF patients with poor ovarian response

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Study question: To test for an increase in one cumulus-oocyte-complex by using a double-lumen needle follicular flushing system (Steiner-Tan®) vs. a 17G single-lumen needle (Gynetics®).
Summary answer: Flushing each follicle 3-times by a modified double-lumen needle does not increase the number of cumulus-oocyte-complexes in poor ovarian response IVF patients.
What is known already: Since the number of oocytes available for IVF is a determinant of pregnancy likelihood, it is paramount to maximize the chance of retrieval of an oocyte from any given follicle, especially in poor responders. Available flushing studies suffer from the use of double-lumen needles with vast dead space resulting in high flow restriction and low flow rate of medium, despite application of high pressure. The Steiner-Tan Needle® is a purpose build quasi-double lumen needle for follicular flushing. The needle, by its specific design, facilitates a high flow rate of flushing medium, which is injected by an automated pump.
Study design, size, duration: Prospective, single-centre, randomized, controlled, open superiority trial comparing a 17G single-lumen aspiration needle (Gynetics®) with the Steiner-Tan Needle® for follicular flushing in patients with ≤5 follicles >10 mm on the day of hCG administration; randomization blocked, allocation concealed; alternative hypothesis: Steiner-Tan Needle® flushing increases oocyte number by one from mean of 3 (estimated SD 1.5); sample size required $n = 80$ (alpha = 5%, beta = 20%, *t*-test, drop-out assumed as 7.5%).
Participants/materials, setting, methods: Patients with a BMI <35 kg/m^2, an indication for IVF/ICSI with or without ovarian stimulation presenting ≤5 follicles >10 mm on day of hCG. In both groups, all visible follicles regardless of size in both ovaries were aspirated. In the study group all aspirated follicles were flushed at least three times. IRB approval (University of Luebeck): EK 2014. Trial registration: 14–244.
Main results and the role of chance: This interim analysis includes the evaluated data after recruitment of 59 patients ($n = 28$ Steiner-Tan Needle®, $n = 31$ Gynetics® aspiration needle *per intention-to-treat*). The analysis *per-protocol* yielded similar results. Demographics and stimulation characteristics were highly similar between groups. The following results were found for the Steiner-Tan® vs. Gynetics® group: punctured follicles 4.1 (±2.3) vs. 4.2 (±1.9) ($p = 0.84$); COCs 2.3 (±1.9) vs. 3.2 (±2.4) ($p = 0.12$); metaphase II oocytes 1.4 (±1.4) vs. 2.1 (±1.5) ($p = 0.07$); 2PN oocytes 0.9 (±1.2) vs. 1.5 (±1.2) ($p = 0.08$); COCs per punctured follicle 0.57 (±0.4) vs. 0.75 (±0.5) ($p = 0.15$); average duration of oocyte-pick-up 3.8 (±2.2) vs. 1.8 (±1.3) min ($p = 0.01$). There is no significant difference in the subjective, peri- and postoperative pain and burden between the study and the control group using the *German Pain Questionnaire score* and the *Depression Anxiety Stress Scale*-21 (DASS 21).
Limitations, reasons for caution: The necessary sample size of 80 patients to reject the hypothesis of superiority has not been achieved at the time of writing. The complete dataset will be ready for presentation at ESHRE.
Wider implications of the findings: If flushing does not increase the number of oocytes, despite the use of an elaborate needle and flushing system, a larger efficiency trial on flushing is redundant, since an increase in the number of oocytes is the only conceivable mechanism of action.
Trial registration number: The trial registration number is NCT02365350.

P-373 The importance of the waist/hip circumference ratio and physical activity on the outcome of intracytoplasmic sperm injection cycles

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Study question: Are the outcomes of intracytoplasmic sperm injection (ICSI) influenced by male and female body mass index (BMI), waist/hip circumference ratio, physical activity and dietary practice?
Summary answer: The female waist/hip ratio and both male and female partner physical activity influence the ICSI outcome. No association was observed concerning BMI and dietary practices.
What is known already: The human fertility rate has declined over time and the underlying cause for this decline has not yet been elucidated. It has been suggested that this decline is associated with many external agents such as environmental factors, as well as eating habits and lifestyle. In both sexes, obesity, particularly abdominal obesity, may impair fertility. A parallel decline in the level of physical activity also exists; however, the evidence of its impact on assisted reproduction outcomes is still weak. It is important to understand which behaviors have the highest negative impact, so that appropriate recommendations can be provided to patients.
Study design, size, duration: This prospective cohort study included 3,913 embryos obtained from 602 couples undergoing ICSI cycles from January 2012 to December 2014, in which embryo transfer was performed on day 5. All patients were interviewed face-to-face by the same nutrition professional before the beginning of the treatment. The questionnaires contained information on weight loss diet, number of meals per day, and frequency of physical activity. The BMI and the waist/hip ratio were also measured.
Participants/materials, setting, methods: The study was performed at a private assisted reproduction centre and enrolled male and female partners. The influences of dietary practice, physical activity, BMI and waist/hip circumference ratio on ICSI outcomes were evaluated using linear and binary regression models. The response variables were: (i) fertilisation rate, (ii) embryo quality on days 2 and 3, (iii) blastocyst formation, (iv) pregnancy, (v) implantation, and (vi) take home baby chance (THB).
Main results and the role of chance: The female waist/hip circumference ratio negatively influenced the fertilisation rate (RC = –0.253, $R^2 = 1.5\%$, $p = 0.015$),

the embryo quality on day 2 (OR = 0.82, CI = 0.67–0.93, p = 0.036) and 3 (OR = 0.79, CI = 0.61–0.89, p = 0.028). No influence of physical activity frequency on any response variable was noted; however, when sedentary patients were compared to physically active patients, positive influences were observed. Physically active women had a higher fertilisation rate (RC = 3.994, R^2 = 1.8%, p = 0.021) and increased chance of high-quality embryos on days 2 (OR = 1.65, CI = 1.23–2.17, p = 0.025) and 3 (OR = 1.52, CI = 1.27–2.02, p = 0.026). Male physical activity did not influence any response variable. Nevertheless, when both the male and female partner were physically active, the chance of blastocyst formation (OR = 2.77, CI = 1.5–3.4, p = 0.037), pregnancy (OR = 1.43, CI = 1.22–1.69, p = 0.041) and THB (OR = 1.09, CI = 1.01–2.4, p = 0.048) were increased. No influence of BMI, being on a weight loss diet or the number of meals per day was noted for any ICSI outcomes.
Limitations, reasons for caution: As lifestyle comprises many factors such as eating habits, stress, and others, it is difficult to infer that a particular factor is the sole cause of reduced or increased ICSI outcomes. Nonetheless, any combination of lifestyle factors could have a significant and cumulative impact on fecundity and warrants due attention.
Wider implications of the findings: Our findings suggest that a lower waist/hip circumference ratio and physical activity have a protective effect on male and female fertility. Awareness about these factors and counseling on how to minimize its impact in each partner could increase the chances of a more favorable rate of conception and live birth.
Trial registration number: N/A.

P-374 Human Papillomavirus (HPV) positive sperm has a negative effect on pregnancy rates in intra-uterine insemination (IUI)

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Study question: Investigate the relationship between HPV infection in sperm and pregnancy outcome after IUI.
Summary answer: Presence of HPV virions detected in sperm is associated with a negative IUI pregnancy outcome.
What is known already: Recent evidence identified HPV-infections as possible cause of male and couple infertility in IUI. In the cervix of infected women, serial measurements of type-specific HPV DNA can categorize HPV-infections in two HPV-induced processes: an infectious virion-producing pathway and a non-infectious transforming or cancer-inducing pathway. Because virions can bind to distinct sites on the spermatozoon's head, this may not only have a detrimental effect on sperm parameters but also on gamete interaction, since *in vitro* experiments have demonstrated that spermatozoa can not only transfer HPV-virions to the oocyte, but the transferred HPV-virions can also induce stage-specific maturation arrest in infected embryos.
Study design, size, duration: Non-interventional prospective study in which different sperm fractions (sperm pellet, swim-up and seminal plasma) were tested on left-over material after IUI for the presence of HPV types (6,11,16,18,31, 33,35,39,45,51,52,53,56,58,59,66,67 and 68) using quantitative real-time PCR.
Participants/materials, setting, methods: The HPV positivity was correlated with IUI outcome and sperm parameters (AML intermediate structure). We calculated that analysis of 1,500 IUI cycli is required to obtain adequate statistical power.
Main results and the role of chance: The HPV prevalence per IUI cycle was 13.6%. Men with HPV-positive sperm were two times less likely to achieve a pregnancy after IUI (5.00 vs. 11.52%; p = 0.0064 for n = 1,500). HPV negative men have significantly more grade A moving sperm (65.3%) than men with HPV (59.4%; p = 0.027).
Limitations, reasons for caution: It is possible that the pregnancy rate per cycle is higher in HPV positive men with HPV negative partners, than in HPV positive men whose partners are also HPV positive. Therefore both partners should be tested for the presence of HPV and ideally each time an IUI cycle is performed.
Wider implications of the findings: Because for HPV multiplicity of infection <10 virions, men can easily infect women. In addition these HPV infected women have longer duration of transient infections resulting in (longer) infertility. Considering the low IUI success rates IVF or ICSI might be indicated in HPV positive couples to increase the pregnancy rate.
Trial registration number: Not applicable.

P-375 Time to empty the bladder after a fresh embryo transfer does not affect pregnancy rates in IVF cycle

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Study question: The influence of the moment of empty the bladder after a fresh embryo transfer (ET) on pregnancy rates in IVF and when is more confortable.
Summary answer: Time to empty the bladder after a ET has no influence on pregnancy rates; delaying it doesn't suppose more success and it is more uncomfortable.
What is known already: Many factors influence the success of assisted reproductive techniques, from both patients and the embryo. Most important factors are maternal age, ovarian reserve, embryo quality, endometrial receptivity and ET technique. To facilitate the ET is necessary that the angle of the uterus is corrected. A full bladder allows it. It has shown that patients with less uterine contractions after ET are more likely to get pregnant and that there are movements of the embryo after ET. It has not shown any action after ET to increase pregnancy rates. We don't know the effect of empty the bladder after ET.
Study design, size, duration: This prospective, randomized, analytical and experimental study included a total of 99 patients from Human Assisted Reproduction Service of our hospital, and was conducted between August 2014 and November 2014. Patients were divided into three groups. Informed consent was signed by patients.
Participants/materials, setting, methods: Patients of our Assisted Reproduction Service with fresh ET were divided in three cohorts at randomization, by an Excel table that allowed a blinded study. A. Empty the bladder after the ET. B. Wait 20 min after ET to empty the bladder. C. Urinary catheterization after the ET. Patients came with full bladder and took rest for 20 min after ET. We compared pregnancy rates and satisfaction (telephone survey). It was analyzed with SPSS.
Main results and the role of chance: No significant differences between biochemical pregnancy rates and clinical pregnancy among the three study groups were found. Biochemical pregnancy rates and clinical pregnancy rates were 30.3%, 32.3%, 28.1% (p = 0.938) and 25% 29% 21.9% (p = 0.807) respectively for cohorts A, B and C. The implantation rate for group A was 0.16, 0.14 for group B and 0.11 for group C (p = 0.763). Patient satisfaction from 0 to 10 by the study groups A, B or C in terms of comfort bladder emptying was respectively: 8.65 ± 1.69, 5.3 ± 3.58 and 7.37 ± 2.69. As we can see, A group scored higher satisfaction.
Limitations, reasons for caution: Live birth rate was not studied. Studies are needed to indicate the live birth rate and larger n in order to obtain greater statistical power.
Wider implications of the findings: The moment of empty the bladder after a ET has no influence in pregnancy rates, so delay it supposes no more success and it is more uncomfortable for patients. Therefore, we should consider it in order to minimize the anxiety and discomfort that this question produces in patients.
Trial registration number: No required.

P-376

Abstract withdrawn by the author

P-377 Subcutaneous progesterone is effective and safe for luteal phase support in IVF: an individual patient data meta-analysis of the phase III trials

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Study question: To summarize efficacy and safety of subcutaneous (s.c.) progesterone as compared to vaginally administered progesterone for luteal phase support in patients undergoing IVF.
Summary answer: No statistical significant or clinical significant differences exist between subcutaneous and vaginal progesterone for luteal phase support.
What is known already: A recent Cochrane review reported that luteal phase support with progesterone is associated with higher rates of live birth or ongoing pregnancy as compared to placebo. Two large phase III studies (07EU/Prg06 and 07USA/Prg05) on s.c. progesterone were finalized in 2013. Both studies were designed and conducted to establish non-inferiority of ongoing pregnancy likelihood in patients undergoing IVF or ICSI and receiving luteal phase support with daily s.c. injections of 25 mg progesterone as compared to vaginally administered progesterone gel or progesterone tablets. Each study showed non-inferiority of s.c. progesterone in relation to vaginal progesterone.
Study design, size, duration: This meta-analysis collates data from two phase III trials (07EU/Prg06, NCT00827983; 07USA/Prg05, NCT00828191) performed according to GCP standards, resulting in a total sample size of 1,483 randomized patients, 1,435 of whom underwent embryo transfer. Outcomes of interest were ongoing pregnancy rate, live birth rate and OHSS risk. Analysis was performed on the level of individual patient data. A comprehensive literature search revealed no further randomized studies on s.c. progesterone usage in IVF.
Participants/materials, setting, methods: A sample size of 1,483 women between 18 and 42 were included in the study. In both studies inclusion criteria were similar, e.g., BMI <30 kg/m^2, <3 prior ART cycles (IVF, ICSI and related procedures), baseline (cycle day 2 or 3) FSH <15 IU/L and E_2 <80 pg/ml, normal uterine cavity as per recent hysterosalpingogram, sonohysterogram or hysteroscopic exam (i.e., no polyp or protruding sub-mucosal fibroid), at least 3 retrieved oocytes and written informed consent.
Main results and the role of chance: The administration of subcutaneous progesterone versus intra-vaginal progesterone had no impact on ongoing pregnancy likelihood (OR = 0.865, 95% CI 0.694–1.077; *P* = n.s.), live birth likelihood (OR = 0.889, 95% CI 0.714–1.106; *P* = n.s.) or OHSS risk (OR = 0.995, 95% CI 0.565–1.754; *P* = n.s.) in regression analyses accounting for clustering of patients within trials, while adjusting for important confounders. Only female age and number of oocytes retrieved were significant predictors of live birth likelihood and OHSS risk.
Limitations, reasons for caution: Licensing studies are conducted in a selected patient population and the external validity of the findings is limited to similar cohorts in daily practice. Furthermore, only IVF cycles with fresh ET were included in the two phase III studies.
Wider implications of the findings: Subcutaneous progesterone 25 mg/day represents a valid alternative to vaginal progesterone and is as efficacious and safe. No further clinical study on the use of s.c. progesterone has been published so far. We expect that the use of s.c. progesterone will be studied in various scenarios, such as frozen-thawed cycles.
Trial registration number: N/A.

P-378 Special considerations for fertility preservation in patients with cervical cancer

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Study question: The aim of this study was to evaluate outcome of cycles and future obstetrical challenges in patients with cervical cancer presenting for fertility preservation.
Summary answer: Even though initial results for outcomes of cycles of oocyte/embryo cryopreservation are promising, long-term results in relation to oncological and obstetrical outcome are still needed.
What is known already: Fertility preserving surgery has been shown to have high success rates in patients with early stage disease. In patients with tumour size ≥2 cm, downstaging with neoadjuvant chemotherapy followed by fertility preserving surgery is being performed, however this is still experimental and long term oncological outcomes are unknown. In this subgroup of patients, fertility preservation by means of oocyte/embryo cryopreservation may be performed, however there has been no previous data assessing outcomes of ART cycles in patients with cervical cancer, particularly with regards to route of oocyte collection with the presence of a cervical mass and future obstetric outcomes.
Study design, size, duration: Retrospective analysis of 15 patients diagnosed to have cervical cancer that presented for fertility preservation between the years 2007 and 2015. Data was obtained from the IVF Hammersmith database.
Participants/materials, setting, methods: Fifteen patients diagnosed with cervical cancer underwent 16 cycles of oocyte and/or embryo cryopreservation. Seven patients presented prior to excision of cervical mass; 4 had laparoscopic oocyte retrieval and 3 had trans-vaginal oocyte retrieval. One patient presented after radical trachelectomy and ovarian transposition and had laparoscopic oocyte retrieval then had a repeat cycle and underwent transvaginal oocyte retrieval from one accessible ovary. Seven patients presented after radical trachelectomy and had trans-vaginal oocyte retrieval of oocytes.
Main results and the role of chance: Mean age at presentation was 30 (range 24–38 years). Histological diagnosis: squamous cell in 13 patients, adenocarcinoma in 1 patient, and large cell neuroendocrine tumour in 1 patient. Stage of cervical cancer at presentation: lB1 in 6 patients, IB2 in 3 patients, IIB in 6 patients. Five patients had laparoscopic oocyte retrieval; mean number of days of stimulation was 9, mean total dose of recombinant FSH was 1,434 IU and mean number of oocytes collected was 6. Seven patients with previous radical trachelectomy had transvaginal oocyte retrieval; mean number of days of stimulation was 11, mean total dose of recombinant FSH was 2,736 IU and mean number of oocytes collected was 15. Three patients had trans-vaginal oocyte retrieval with presence of cervical mass; mean number of oocytes collected was 15 and cancer free period to date is 50 months, 32 months and 12 months respectively. Ten patients had embryo cryopreservation, 2 had oocyte cryopreservation and 2 had oocyte and embryo cryopreservation. Two patients had three cycles of frozen embryo replacement cycles in a surrogate, all of which did not result in a pregnancy. One patient had an elective single embryo transfer post trachelectomy that resulted in a clinical pregnancy.
Limitations, reasons for caution: This is a retrospective analysis of our database and sample size is small, hence not enough data is available to evaluate safety and possible recurrence rates when transvaginal oocyte retrieval is performed with the presence of a cervical mass. Data is also insufficient to conclude on future pregnancy outcomes.
Wider implications of the findings: Transvaginal oocyte retrieval with the presence of a cervical mass is a novel procedure, and in experienced hands may be performed safely and yield a higher number of oocytes than laparoscopic oocyte retrieval. Patients need to be aware about the future need for a surrogate prior to undergoing this.
Trial registration number: N/A.

P-379 Predicting the success of IVF: external validation of the van Loendersloot's model

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Study question: Is the predictive model for IVF success proposed by van Loendersloot et al. valid in a different geographical and cultural context?
Summary answer: The model discriminates well but less than in the original context where it was developed.
What is known already: Several independent groups have developed models with the aim of estimating the chance of pregnancy with IVF but only four of them were externally validated. One of these, the van Loendersloot's model, deserves particular attention and further investigation for at least three reasons: 1) the Area under the receiver operating characteristics curve (*c*-statistics) in the temporal validation setting was the highest reported to date (0.68), 2) the perspective of the model is clinically wise since it includes variables obtained

from previous failed cycles. It thus adapts to any woman entering an IVF cycle, 3) the model lacks geographical external validation.

Study design, size, duration: Retrospective cohort study of women undergoing oocytes retrieval for IVF between January 2013 and December 2013 at the infertility unit of the Fondazione Ca' Granda, Ospedale Maggiore Policlinico of Milan, Italy. Women were enrolled only for their first oocytes retrieval cycle performed during the study period. They were excluded if they underwent previous IVF cycles in other centers. The main outcome was the cumulative live birth rate per oocytes retrieval.

Participants/materials, setting, methods: Seven hundred seventy-two women were selected. Variables included in the van Loendersloot's model and the relative weights (beta) were used. The variable resulting from this combination (Y) was transformed into a probability. The discriminatory capacity was assessed using the *c*-statistics. Data is presented using both the original and the calibrated models obtained with a logistic regression. Performance was evaluated correlating the mean predicted chances of live births in the five quintiles and the observed rates.

Main results and the role of chance: Two-hundred-eleven live births (27%) were obtained. The *c*-statistics was 0.64 (95%CI: 0.61–0.67, $p < 0.001$). The slope of the linear predictor (calibration slope) expressed as an Odds Ratio was 1.81 (95%CI: 1.46–2.24, $p < 0.001$), corresponding to a beta of 0.630. The calibration intercept was +0.349 ($p = 0.13$). While a clear discrepancy exists using the original model, data appears properly distributed with the calibrated model. The Pearson coefficient of the correlation between the mean predicted chances of live births in the five quintiles and the observed rates was 0.99 ($p = 0.002$).

Limitations, reasons for caution: The selection criteria for access to IVF adopted in our center might be too stringent, leading to the exclusion of women with yet acceptable chances of live birth. The validity of the model in women at very low chance of live birth could not thus be tested.

Wider implications of the findings: The van Loendersloot's model can be used in other contexts but needs important calibration. It may be of help for counselling couples about their chance of success but it cannot be used to decline treatments. Further research is needed to improve the discriminatory performance of IVF predictive models.

Trial registration number: Not applicable.

P-380 Utility of three-dimensional ultrasound (3D-US) in screening for subtle septum in infertile women with arcuate uteri prior to IVF: a scenario economic analysis

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Study question: Is utility of 3D-US a cost-effective screening strategy for subtle septae in infertile women with hysterosalpingographic (HSG) diagnosis of arcuate uterus prior to IVF?

Summary answer: Prior to IVF, 3D-US screening for subtle uterine septae in infertile women with HSG diagnosis of arcuate uterus seems to be a cost-effective strategy.

What is known already: Current evidence suggests that, unlike arcuate uterus which has no effect on implantation, small and large uterine septae could be deleterious. Increase in live birth rate (LBR) has been reported after resection of uterine septae prior to IVF. Subtle septae have been reported by recent studies in women with HSG diagnosis of arcuate uterus who had prior failed IVF. These septae are diagnosed by office hysteroscopy after repeated IVF failure. Yet, no studies have addressed the value of 3D-US screening for subtle septum prior to first IVF cycle in this group of IVF women in terms of LBR and economics.

Study design, size, duration: A decision analysis model was constructed for a hypothetical cohort of 100 infertile women with HSG diagnosis of arcuate uterus scheduled for first IVF cycle, to compare the cost of 2 scenarios (strategies) over 2 successive IVF cycles; (1) Screening for subtle septum by 3D-US prior to IVF. (2) Proceeding to IVF without 3D-US screening. (No 3D-US strategy). Baseline input probabilities were derived by review of the published literature.

Participants/materials, setting, methods: Prevalence of misdiagnosed septate uterus in our hypothetical cohort, and the effect of hysteroscopic metroplasty on LBR were obtained from the available evidence in literature. Medicare National Fee Estimates were considered for costs assumptions. Study endpoints included cumulative LBR and the incremental cost-effectiveness ratio (ICER) after 2 IVF cycles. Costs were reported in 2015 US dollars. Sensitivity analysis was performed, to examine the effect of variation of the baseline inputs.

Main results and the role of chance: Screening for subtle uterine septum in women with hysterosaplingographic diagnosis of arcuate uterus prior to IVF was more cost effective than No 3D-US strategy. After 2 IVF cycles, 3D-US screening strategy resulted in cumulative LBR, costs per one LB and costs per infertile couple of 48%, $45,872 and $22,019, respectively compared to 33%, $73,310 and $24,192 in No 3D-US strategy. 3D-US implementation would cause initial extra-costs of $26.900; however, this would save extra-costs of $217,351 required by women with failed IVF for additional IVF cycles and additional office hysteroscopy evaluation of their uterine cavities to detect the missed septum as a cause of IVF failure. In No 3D-US strategy, the extra costs for achieving an additional LB; the ICER was $14,490. Sensitivity analyses were robust.

Limitations, reasons for caution: Hypothetical rather than actual estimates of cost present a limitation of this study**.**

Wider implications of the findings: Adoption of 3D-US, to reevaluate arcuate uteri initially diagnosed by HSG prior to IVF, could minimize the financial burden that infertile couple will suffer from failed cycle due to missed septum. This strategy may be helpful, since randomized controlled trials in IVF women face recruitment challenges.

Trial registration number: NA.

P-381 Iron and vitamin status in Italian female patients undergoing *in vitro* fertilization (IVF)

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Study question: To evaluate homocysteine and related B-vitamins, red-blood-cells (RBC) folate, vitamin A, vitamin E and iron status in a population of women undergoing *in vitro* fertilization.

Summary answer: A large proportion of women had RBC folate, vitamin B12, vitamin A deficiencies. Homocysteine was above adequate levels in less than one third of women.

What is known already: Biochemical indicators, including vitamins and iron-status indicators can be important to assess the health and nutrition status of specific populations. Nutrition deficiencies vary by age, gender or race/ethnicity and could be extremely relevant for certain population groups like childbearing women because they can influence the outcome of pregnancy and/or the health of the baby. Iron deficiency is the most common nutrient deficiency in the developed world and women of childbearing age are at greatest risk because of the effects of menstruation.

Women seeking a pregnancy are generally encouraged to receive adequate nutrition, and multivitaminic/multimineral supplementation.

Study design, size, duration: This is a cross-sectional study of 269 caucasian women aged 18–45 consecutively scheduled for IVF. Each identified subject was interviewed about her medical history and vitamin/mineral dietary supplements. Recruitment period lasted from March to December 2015.

Participants/materials, setting, methods: During routine blood sample examination before IVF treatment an additional amount of fasting venous blood

was drawn in order to measure: folate, RBC folate, homocysteine, vitamin B12, vitamin A, vitamin E, iron and ferritin. Samples were immediately taken to the laboratory to be processed within 2 h (vitamin B12, homocysteine, iron and ferritin) or stored at −20°C until assayed (serum folate, RBC folate, vitamin A and vitamin E).

Main results and the role of chance: The mean age of the enrolled women was 37 ± 4 (range 27–45) years; the mean BMI was 22.3 ± 4.3 (range 15.2–51.1 Kg/m^2). Percentages [95%CI] of women reaching concentration levels considered appropriate for the general population (reported between brackets) together with mean ± standard deviation values were: Serum folate (>6.6 ng/ml): 78% [72-83%]; 11.4 ± 5.4 ng/ml RBC folate (>225 ng/ml): 74%[69–79%]; 293 ± 91 ng/ml. Homocysteine (<10 mM/L): 69% [64–75%]; 9.4 ± 4.0 mM/L. Vitamin B12 (>474 pg/ml): 44% [38–50%]; 476 ± 216 pg/ml. Vitamin A (>0.4 mg/L): 44% [37–49%]; 0.40 ± 0.10 mg/L. Vitamin E (>5 mg/ml): 100% [99–100%]; 12.4 ± 2.3 mg/ml. Serum iron (>60 mcg/dl): 83% [78–88%]; 93.0 ± 36.5 mcg/dl Serum ferritin (20–200 mg/L): 82% [77–87%]; 53.0 ± 39.4 mg/L. When considering the RBC folate threshold suggested to achieve the greatest reduction of NTDs (400 ng/ml), the percentage of women with adequate levels dropped to 12% [8–16%]. The use of folate/vitamin supplements significantly (p = 0.001) increased the percentage of women with adequate levels of serum folate (94% [89–97%] versus 64% [55–72%]), RBC folate (22% [15–31%] versus 3% [1–7%]), homocysteine (83% [77–90%] versus 56% [48–65%]) in users compared to non users, respectively.

Limitations, reasons for caution: Reference values for most vitamins are matter of debate. We mainly refer to thresholds used in the Italian population. Different thresholds may significantly modify the scenario. Moreover, caution is needed for inferences to other contexts. Dietary habits influence biochemical indicators and differ according to social/medical conditions, ethnicity, region/country of provenance.

Wider implications of the findings: Our results highlight relevant deficiencies in iron and vitamin status of women initiating IVF. Of note, a small minority of women showed optimal RBC-folate levels. Given the role of folate in the prevention of important diseases, more effective public health strategies are needed to increase the use of dietary supplements.

Trial registration number: NA.

P-382 Type of gonadotropin does not affect either oocyte quality or follicular fluid endocrine profile during ovarian stimulation in oocyte donors

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Study question: Is it possible to stimulate with any gonadotropin without affecting oocyte quality in a donor program?

Summary answer: Results from endocrine profile and apoptosis rate suggest that type of gonadotropin does not affect oocyte quality when stimulating a donor.

What is known already: Type of gonadotropin determines FSH levels in serum and follicular fluid, based in the proportion of acid residues in the FSH molecules. This should be considered due to gonadotropins are the main physiological regulatory hormones of follicular survival because of their anti-apoptotic properties; in addition, sex steroids act as an important regulators of intra-ovarian follicular atresia, meaning that an increased amount of estrogens are related to reduced apoptotic activity.

Study design, size, duration: A multicentre prospective randomized study was performed in 3 private clinics belonging to IVI group. Oocyte donors (n = 215) undergoing their first stimulation cycle were allocated to a stimulation group with recombinant FSH, highly-purified human menopausal gonadotropin HP-hMG or corifollitropin alfa. Blood samples were obtained on the day of hCG administration for estradiol (E2) analysis. Apoptosis rate was determined by flux cytometry in cumulus cells and hormonal profile in follicular fluid, both obtained at oocyte pick-up.

Participants/materials, setting, methods: Subjects were assigned to receive 100 µg of corifollitropin alfa which was potentially followed by daily administration of recombinant FSH (rFSH) from day 8 if instructed by the researcher (n = 67), or daily doses of 150 UI rFSH (n = 73) or 225 UI HP-hMG (n = 75). Daily doses of 0.25 mg GnRH antagonist were started on day 6 of stimulation in all groups. A single dose of GnRH agonist (0.2 mg) was administered for triggering final oocyte maturation.

Main results and the role of chance: According endocrine profile in follicular fluid, FSH concentration were significantly lower in the rFSH group (5.00 UI/L) vs. corifollitropin alfa (7.11 UI/L) or HP-hMG (7.15 UI/L), p = 0.05, while estradiol concentration were significantly higher in the HP-hMG group (1,885,545 pg/ml) compared to recombinant FSH (396,582 pg/ml) and corifollitropin alfa (597,398 pg/ml), p = 0.002. In serum, we found a significant higher estradiol concentration in the group stimulated with HP-hMG (3,643 pg/ml) compared with rFSH (1,542 pg/ml) and corifollitropin alfa (1,456 pg/ml), $p < 0.001$. Apoptosis rate in cumulus cells did not differ between all the study groups being the results as follows: 26.5% in the rFSH group, 26.2% in the corifollitropin alfa stimulation and 24.1% in the HP-hMG group, p = 0.840

Limitations, reasons for caution: This study has been performed in oocyte donors, which form a fairly homogeneous group in terms of age and ovarian response, so these results may not be extrapolated to other groups of women undergoing an assisted reproductive treatment.

Wider implications of the findings: Despite of the higher concentrations of FSH observed in follicular fluid from donors stimulated with HP.hMG or corifllitropin alfa, probably due to an acidic and longer half-life FSH, the absence of differences in apoptosis rate means the possibility of stimulating oocyte donors with any drug without compromising clinical results.

Trial registration number: NCT02213627

P-383 Effect of *in vitro* and *in vivo* acrylamide exposure on mouse oocyte

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Study question: Does acrylamide have harmful effects on *in vitro* and *in vivo* oocyte maturation?

Summary answer: Acrylamide blocks *in vivo* oocyte maturation by its metabolite, glycidamide, but it does not affect *in vitro* oocyte maturation.

What is known already: Acrylamide is a chemical that widely used in the treatment of wastewater, paper/pulp manufacturing, mining and scientific research. Processed foods such as roasted almonds, coated peanuts, dips, fried potato, biscuits, coffee, contain high levels of acrylamide. The consultation of the United Nations Food and Agricultural Organization and the World Health Organization on "Health Implications of Acrylamide in Food", indicated several important points such as; dietary habits in particular countries may lead to severe carcinogenic implications, as well as genotoxicity. Acrylamide effects on the oocyte maturation and meiotic spindle still need to be evaluated.

Study design, size, duration: BALB/c mice were used. *In vitro* and *in vivo* maturation kinetics of acrylamide treated oocytes (n = 182 and n = 133, respectively) were compared to control group (n = 77 and n = 102). *In vitro* maturation dynamics of glycidamide treated oocytes (n = 61) were compared to control oocytes (n = 24). Meiotic spindles were evaluated under a confocal microscope following fluorescently labeling the meiotic chromosomes and spindle microtubules.

Participants/materials, setting, methods: *In vitro* maturation kinetics of control oocytes were compared to 100, 500 or 1,000 µM of acrylamide treated ones. *In vivo* maturation analyses were performed following oocyte isolation from the animals that were intraperitoneally treated with either vehicle or 25 mg/kg/day of acrylamide for 7 days. To test whether *in vivo* effects of acrylamide resulted from its metabolite glycidamide or not, isolated oocytes were treated with 25 or 250 µM of glycidamide.

Main results and the role of chance: *In vitro* acrylamide exposure did not affect *in vitro* oocyte maturation and meiotic spindle. *In vivo* acrylamide

treatment caused an accumulation of oocytes at prometaphase-I (10.3% vs. 2.3% in controls, $p = 0.039$). Majority of the M-II stage oocytes from *in vivo* acrylamide treated group showed a decrease in the meiotic spindle mass and a misalignment of the chromosomes. This finding indicated that acrylamide might show its action via its metabolite glycidamide. Therefore, isolated GV-stage oocytes treated with 25 or 250 µM of glycidamide *in vitro*. All oocytes were degenerated after the culture period.
Limitations, reasons for caution: This study presents the outcomes of an animal model. It will be a good contribution if the effects of acrylamide or glycidamide were evaluated in human (discarded) oocytes that can be obtained from *in vitro* fertilization laboratories. Additionally, long and short terms reproductive effects of acrylamide need to be evaluated.
Wider implications of the findings: Dietary habits have been changing in parallel with the modern life. The finding, which acrylamide negatively affects oocyte maturation, suggests that it may reduce fertilization and implantation rates and could cause aneuploidy in higher doses, and thus implies that changing dietary habits must be reconsidered regarding female fertility.
Trial registration number: N/A.

P-384 The use of donor sperm in addition to donated oocytes after repeated implantation failure with donated oocytes does not improve live birth rates

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Study question: Does switching to donor sperm in a subsequent cycle after repeated implantation failures in several cycles with donated oocytes increase the live birth rate?
Summary answer: Switching to donor sperm after several implantation failures in cycles with donated oocytes and partner sperm does not increase live birth rate.
What is known already: About 2% of patients receiving donated oocytes experience repeated implantation failures, despite normal anatomy of the uterine cavity and a diagnostic biopsy dismissing endometrial hyperplasia or endometritis, normal sperm characteristics, normal male karyotype, and a normal thrombophilia panel (antithrombin III activity, protein "S" coagulation inhibitory activity, anti-microsomal antibodies, antibody antitireoglobulin thyrotropin, lupus anticoagulants, protein "C" coagulation inhibitory activity, anticardiolipin IgM and IgG antibodies, resistance to activated protein C, homocysteine, prothrombine mutation 20,210). The ESHRE Capri Workshop Group indicates that donated semen can be offered after 3 failed cycles in order to improve reproductive outcomes, without evidence of male factor.
Study design, size, duration: Retrospective cohorts study including cycles with donated oocytes and either normozoospermic partner sperm (OD) or with donation of both oocyte and sperm (DD), performed following at least 3 oocyte reception cycles with implantation failure, performed between January 2011 and December 2014 in a large fertility center.
Participants/materials, setting, methods: The study included 228 cycles, 159 in OD group and 69 in DD group. The main outcome was live birth rates, analyzed with both a univariate and a multivariate approach, adjusted for the oocyte recipient's age and body mass index (BMI), day of embryo transfer, number of transferred embryos and embryo quality. Secondary outcomes were biochemical, clinical, and ongoing pregnancy rates.
Main results and the role of chance: There was no difference in live birth rate between the DD and OD groups (38.2% vs. 35.8%, $p = 0.73$), even after adjustment for confounding factors (OR 1.41, 95%CI 0.72, 2.75; $p = 0.31$). Rates of biochemical pregnancy (52.2% vs. 54.1%, $p = 0.79$), clinical pregnancy (41.2% vs. 45.9%, $p = 0.51$) and ongoing pregnancy (38.2% vs. 37.1%, $p = 0.87$) were similarly not different between the DD and the OD groups. The DD and OD groups were comparable at baseline in all demographic and cycle variables analyzed (recipient's BMI, number of transferred embryos and embryo quality) with exception of the recipient's age (42.3 in DD vs. 44.1 in OD), and day of embryo transfer (56.5% of DD and 83.6% of OD embryo transfers were performed on blastocyst stage); both variables were adjusted for in the multivariate analysis.
Limitations, reasons for caution: The main limitation of this study is its retrospective nature, and the lack of extensive molecular testing of sperm in normozoospermic patients. This last limitation is significantly attenuated by the lack of improvement in reproductive outcomes when changing partner semen for donor semen.
Wider implications of the findings: In cases of repeated implantation failure with donated oocyte and normozoospermic sperm, switching to donor sperm should not improve results. In disagreement with currently published guidelines, the physician should probably not offer the change to donor's sperm without a clinical indication of male factor infertility.
Trial registration number: NA.

P-385 Time from egg retrieval to embryo transfer does not affect live birth rates in a freeze-all strategy

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Study question: Does time from ovum pick-up (OPU) to frozen embryo transfer (FET) affect live birth rates in a freeze-all strategy?
Summary answer: Performing FET on the first or subsequent menstrual cycles after OPU does not affect live birth rates (LBR) in a freeze-all strategy.
What is known already: Controlled ovarian stimulation (COH) leads to altered endometrial gene expression patterns, histological advancement, and decreased reproductive outcomes by affecting the embryo-endometrium synchrony. There's little information regarding the amount of time it takes for gene expression to return to its normal pattern. Moreover, delaying FET may have a significant emotional impact on patients, which adds up to the stress and anxiety that accompanies a standard IVF cycle. So far, there is no agreement on the best moment to perform a FET after freeze-all in order to maximize reproductive outcomes for the patient.
Study design, size, duration: Retrospective, cross-sectional study of 680 freeze-all cycles, corresponding to 649 women, performed between January 2012 and December 2014. COH was performed by either a GnRH-antagonist or a long GnRH-agonist protocol. Ovulation was triggered using either a GnRH-agonist ($n = 357$) or hCG ($n = 323$). Endometrial preparation was performed by either a modified natural ($n = 117$) or an artificial cycle ($n = 563$).
Participants/materials, setting, methods: Reproductive outcomes between FET who took place within the first menstrual cycle following OPU ($n = 324$) or afterwards ($n = 356$) were compared. Student's *t*-test for independent samples and Chi-square analysis were used as needed. A multivariate logistic regression analysis was performed adjusting for maternal age, drug used for ovulation trigger, number of retrieved oocytes, number of embryos obtained, day of embryonic development at transfer, and number of transferred embryos. Differences were considered significant if $p < 0.05$.
Main results and the role of chance: There was no difference in live birth rate between FET performed during the first menstrual cycle following OPU vs. subsequent menstrual cycles (36.4% vs. 32.3%, respectively; $p = 0.258$). Moreover, we found no difference in implantation rate (32.5% vs. 29.5%; $p = 0.289$), as well as biochemical pregnancy (49.7% vs. 48.3%; $p = 0.719$), clinical pregnancy (43.5% vs. 41.3%; $p = 0.555$), and pregnancy loss (13.3% vs. 15.7%; $p = 0.363$) between FET performed during the first menstrual cycle following OPU vs. subsequent menstrual cycles. A multivariate analysis found no impact of timing of elective FET on live birth rates (OR 0.87; 95% CI 0.62–1.21). The impact remained not significant after adjusting for drug used for ovulation trigger (hCG vs. GnRH agonist) and endometrial preparation protocol (natural vs. artificial). The factors that significantly affected live birth rates were, as expected, maternal age (OR 0.93, 95% CI 0.89–0.97), number of retrieved oocytes (OR 1.03, 95% CI 1.001–1.06), day of embryonic development at transfer (day + 3 vs. + 4; OR 1.53; 95% CI 1.02–2.29) and number of transferred embryos (OR 1.75, 95% CI 1.25–2.46).
Limitations, reasons for caution: The main limitation of our study is its retrospective nature. Although we adjusted our statistical analysis for a number of known and suspected confounders, we cannot exclude the possibility of residual confounding factors.
Wider implications of the findings: According to our results, clinicians do not need to wait more than one menstrual cycle before performing FET. This allows

us to reduce the psychological impact that delaying embryo transfer can have on our patients, without compromising reproductive outcomes.
Trial registration number: NA.

P-386 Concordance of vitamin D levels in the infertile couples' partners

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Study question: Do vitamin D serum levels correlate in infertile couples' partners?
Summary answer: Serum Vitamin D levels highly correlate between the partners of infertile couples.
What is known already: There is emerging evidence suggesting a role of insufficient vitamin D in both male and female infertility. However, until now, available studies have focussed separately on the male or female partners. Given the crucial role of dietary and lifestyle habits in influencing peripheral levels of vitamin D, one may conversely hypothesize that levels may significantly correlate within couples' partners. In other words, vitamin D insufficiency may display its detrimental effects through both partners. However, evidence in favour of a correlation of vitamin D status between the partners of infertile couples is lacking in the literature.
Study design, size, duration: Between March and May 2014, couples referred to the Infertility Unit of the Fondazione Ca' Granda for IVF were consecutively considered for study entry. Initial selection criteria were as follows: female age 18–42 years and female body mass index (BMI) ≤25 Kg/m2. Exclusion criteria for both partners were a history of malignancy, hypertension, diabetes, multiple sclerosis, need for chronic medical treatments, autoimmune disorders and coronary, hepatic or renal diseases.
Participants/materials, setting, methods: Eligible couples accepting to participate provided a serum sample for 25-hydroxy-vitamin D [25(OH)D] measurement during the routine clinical assessment that preceded the initiation of an IVF cycle. The quantitative detection of total 25(OH)D levels was performed on fresh samples using a commercially available kit based on a chemiluminescence technology. Subjects were grouped according their serum levels of vitamin D, namely: "insufficient" when 25(OH)D was <20 ng/ml or "sufficient" when it was ≥20 ng/ml.
Main results and the role of chance: One hundred and three eligible couples were included. The mean ± SD 25(OH) levels were 16.4 ± 6.2 and 17.2 ± 6.0 ng/ml, in female and male partners, respectively ($p = 0.17$). They were significantly correlated, with a Pearson's r = 0.52 ($p = 0.001$). The number of women with 25(OH)D serum levels <20 ng/ml and ≥20 ng/ml were 73 (71%) and 30 (29%), respectively; similarly 77 (75%) men resulted insufficient for vitamin D level while 26 (25%) resulted sufficient. In 13 (13%) couples both partners showed sufficient vitamin D levels while in 60 (58%) couples both partners were classified as "insufficient"; on the contrary, 17 (16%) couples had "sufficient" female and "insufficient" male partner and 13 (13%) couples had male with 25(OH)D level ≥20 ng/ml and female <20 ng/ml. The contingency table highlighted an overall concordance rate equal to 73/103 (71%) compared to 30/103 (29%) couples showing discordance ($p = 0.007$). Comparing baseline characteristics between female with insufficient and sufficient vitamin D levels we did not found any differences with the exception for the presence of endometriosis, which is more frequent in the sufficient group. We failed to highlight any differences in sperm basal characteristics between men with sufficient and insufficient vitamin D levels.
Limitations, reasons for caution: The present study has been designed to describe vitamin D correlation between infertile requiring IVF. Data from the more general population of infertile couples is warranted. Moreover, the chances of pregnancy according to couple' vitamin D status were not investigated. Lastly, we did not investigate spousal concordance over time.
Wider implications of the findings: The study confirmed the high rate of vitamin D insufficiency in infertile patients and showed a high concordance between partners of IVF couples. Considering the possible effects of vitamin D on infertility, supplemental strategies involving both partners rather than only one may be wiser and deserve future investigations.
Trial registration number: Not applicable.

P-387 Expressions of aquaporin family in human luteinized granulosa cells and their correlations with IVF outcomes

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Study question: Are mRNA for specific aquaporins (AQPs) expressed in human luteinized granulosa cells (GCs) and their expression levels correlated with *in vitro* fertilization (IVF) outcomes?
Summary answer: AQP1-7, 9, 11, and 12 was expressed in luteinized-GCs and AQP1 was negatively associated with retrieved-oocyte number and AQP7 was positively associated with fertilization rate.
What is known already: Currently, mRNA of AQP1–4 and AQP9 has been reported to be present in human GCs.
Study design, size, duration: Prospective observational study involving 111 women undergoing a stimulated IVF cycle.
Participants/materials, setting, methods: Luteinized GCs were obtained at the time of oocyte retrieval. The mRNA expressions of AQP0-12 were explored by RT-PCR in GCs from another 27 women, and mRNAs for AQP0, 8 and 10 were not detected. Real-time quantitative RT-PCR (qRT-PCR) was performed to quantify mRNA level of AQP1-7, 9, 11, and 12. The mRNA for luteinizing hormone receptor (LHR) and steroidogenic acute regulatory protein (StAR) were also quantified by qRT-PCR.
Main results and the role of chance: In samples from 111 women, retrieved oocyte number was negatively associated with the mRNA levels of AQP1, 4, 6, and 11 and LHR (r = –0.311, r = –0.233, r = –0.203, r = –0.194, and r = –0.202, respectively, $p < 0.05$ for each), however, after adjustment for woman's age and serum anti-müllerian hormone (AMH) levels, only correlation with AQP1 was found (r = –0.299, $p < 0.05$). Body mass index was negatively associated (after adjustment of age) with the mRNA level of AQP7 (r = –0.259, $p < 0.05$). Fertilization rate was positively associated with the mRNA level of AQP7 (r = 0.269, $p < 0.05$). The number or quality of embryos or clinical pregnancy was not associated with the mRNA levels of any of ten AQP subtypes. The mRNA levels for the ten AQP subtypes were correlated positively with LHR expression but negatively with StAR expression. Amongst high responders (oocyte number ≥14), the mRNA levels of AQP11 (1.4 ± 0.7 versus 1.7 ± 0.6) and LHR (1.3 ± 0.7 versus 1.7 ± 0.7) were significantly lower in the group with PCOS than in the non-PCOS group.
Limitations, reasons for caution: A relative small number of subjects in PCOS group would be the main limitation of our study.
Wider implications of the findings: AQP1 may be one of the factors that modulate individual ovarian response to exogenous gonadotropin. The mRNA level of AQP7 was positively associated with fertilization rate, which is a surrogate marker of oocyte competence, thus expression of AQP7 could be a marker for adequate folliculogenesis and healthy oocytes.
Trial registration number: No RCT.

P-388 Oocyte donation from a family-member

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Study question: Does oocyte donation from a family-member improve success rates compared to that from an unrelated donor?
Summary answer: Oocyte donation from a family member or from an unrelated donor is associated with comparable rates of pregnancy and live birth.

What is known already: Oocyte donation is on the rise, mostly due to increasing number of older infertile women. In many countries, commercial oocyte donation is prohibited and paying the donor is illegal. Accordingly, recipients are required to find a donor to donate oocytes altruistically. Oocyte donation from a family member and especially from a sibling may be the most available option, and the ASRM ethics committee approves sister-to-sister oocyte donation. To date, there have been only several publications evaluating family member oocyte donation; they focused mainly on the psycho-social aspect and not on the clinical outcome.
Study design, size, duration: A retrospective cohort study performed at a single academic reproductive center.
Participants/materials, setting, methods: We compared the outcome of oocyte donation cycles from a family member (*n*: 123 cycles) and from an unrelated donor (298 cycles) conducted between 2010 and 2014. Subgroup analysis comparing the outcomes using sibling donor and unrelated donor was also performed.
Main results and the role of chance: Recipient basal FSH levels were lower in the family-member donor group (27.2 ± 27.1 IU/L) than in the unrelated donor group (38.0 ± 36.7 IU/L, *P*: 0.002, 95% CI −17.6; −4.1). More recipients in the non-family member group were menopausal (26.1% vs. 14.6%, $p = 0.01$ CI −0.2; −0.03). Family-member donors had higher previous parity (1.52 ± 1.55) than non-related donors (1.02 ± 1.14, $p = 0.02$ CI −0.6; −0.06). All other demographic characteristics as well as the cycle profiles were comparable. The rates of pregnancy, live birth and miscarriage rates were similar between the family member donor group and the unrelated donor group. There were 57 cycles of sibling donation (sister to sister). Sibling donors were older (33.9 ± 5.1 vs. 29.9 ± 5.3, $p < 0.0001$ CI 2.2;5.7) and had lower AFC (16.21 ± 10.2 vs. 21.1 ± 13.9, $p = 0.02$ CI −8.9; −0.8) than the unrelated donors, but the rates of pregnancy and live birth were not significantly different (42.9% and 22.8% in sibling-donor group vs. 50.3% and 22.8% in the non-related group).
Limitations, reasons for caution: Retrospective study.
Wider implications of the findings: Oocyte donation from sisters or other family members achieves comparable results compared to that from unrelated members. The former may be less expensive and more satisfying to the recipients. Oocyte donation from family members may be an acceptable option especially in countries where commercial donation is prohibited.
Trial registration number: N/A.

P-389 Oocyte cryopreservation for social reasons: a two-center study of the users' reproductive situations

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Study question: With more women seeking oocyte cryopreservation (OC) for delaying childbearing (social reasons), little is known related to their reproductive situations after the procedure.
Summary answer: Only one fifth of the users attempted pregnancy after OC and only 8% of the users ever returned to use their frozen oocytes.
What is known already: A small number of studies from the USA and Europe have provided some data on the profile and characteristics of women who have undergone OC for social reasons. However, information about these users' later-on reproductive situations and the outcome of the cryopreserved oocytes were limited.
Study design, size, duration: This is a retrospective cohort study, analyzing the reproductive situations of the women having undergone social OC after at least one year follow-up. Totally 235 women underwent social OC from 2002 to 2014. They were contacted by phone to evaluate their reproductive situation by a senior staff in the Stork Fertility Center or by a senior fellow in National Taiwan University Hospital. Women who were not reached or who refused to respond were excluded.
Participants/materials, setting, methods: There were 178 women going through the interview completely. The response rate was 75.7%. Their reproductive situations were categorized to four subgroups: (1) women attempting pregnancy naturally (2) or from frozen-thawed oocytes, (3) women who did not try to conceive despite there's a stable relationship (4) or because they were single/without stable relationship. Their demographic data and the clinical results of the frozen-thawed oocytes were analyzed.
Main results and the role of chance: Among those who underwent the interview completely, the mean age at social OC was 38.3 ± 3.7 years old with a mean of 10.2 ± 7.6 oocytes frozen per person. The majority were women who did not attempt pregnancy (138 women, 77.5%), 106 (59.6%) women were single or without stable relationship while 32 (18%) women had stable relationship. Only 25 (14.0%) women attempted pregnancy naturally, and 14 women (7.9%) returned to thaw their oocytes. One woman conceived from fresh IVF/ET instead of using cryopreserved oocytes for personal favor. Women who did not attempt pregnancy despite there's a stable relationship tended to stored their oocytes earlier than other subgroups, and even significantly earlier than women who were single or without stable relationship (36.4 ± 4.4 vs. 38.8 ± 3.4 years old, $P = 0.002$). Whereas women who attempted pregnancy naturally tended to be younger than those from frozen-thawed eggs in the aspects of the age at OC (38.4 ± 4.0 vs. 39.5 ± 4.1 years old) and the age with ongoing pregnancy (37.3 ± 3.0 vs. 39.2 ± 4.8 years old). The ongoing pregnancy rates of frozen-thawed oocytes from different ages at OC (≤35 years old: 100%, 36~39 years old: 50%, ≥40 years old: 25%) seemed similar to those of fresh oocytes in our fertility centers.
Limitations, reasons for caution: At least one year follow-up may be too short to evaluate the reproductive situations for some users. The population who thawed their oocytes is not big enough for a more convincing pregnancy outcome. There might be some information bias as a result of the retrospective aspect.
Wider implications of the findings: The value of social OC for fertility preservation remained unclear. We noted only 21.9% of the users ever attempted pregnancy, and 7.9% returned to use the frozen oocytes. Hope more study with longer follow up will be reported.
Trial registration number: Nil.

P-390 Lack of association between smoking habit at the time of treatment and live birth in recipients of oocyte donation cycles

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Study question: Is smoking habit in donors, recipients, and male partners associated with live birth in oocyte donation cycle?
Summary answer: Smoking is not associated with a compromised oocyte quality or uterine receptiveness in oocyte donation cycles, as live birth rate remains unaffected by smoking.
What is known already: Smoking has been shown several times to have deleterious effects on female fertility and on autologous *in vitro* fertilization (IVF) outcome, but very little is known on its respective impact on oocyte quality and uterine receptiveness. A single previous investigation in oocyte donation cycles has suggested that smoking in recipients was associated with lower pregnancy rate in heavy smokers than in non-heavy smokers.
Study design, size, duration: This is a retrospective cohort study encompassing oocyte donation cycles with partner sperm performed between 2010 and 2014 in a large fertility center. A total of 7,470 donors and 9,747 recipients undergoing 12,121 oocyte donation cycles were included.
Participants/materials, setting, methods: Smoking habit was categorized for each party as follows: Non-smokers (NS), light smokers (LS; 1–10 cigarettes/day) and Heavy smokers (HS; >10 cigarettes/day). The main outcome of the study was live birth, which was analyzed by univariate and multivariate analysis, adjusted for education level, working status, day of embryo transfer, number of transferred embryos, and embryo quality. Secondary outcomes were biochemical, clinical, and ongoing pregnancy.
Main results and the role of chance: Live birth rates were not affected by degree of smoking in either donor (OR 1.1 [0.99–1.24] and 1.1 [0.96–1.25] in LS and HS, respectively), recipient (OR 0.99 [0.85–1.16] and 1.0 [0.79–1.31] in LS and HS, respectively) or recipients' partner (OR 1.0 [0.86–1.16] and 1.1

[0.95–1.32] in LS and HS, respectively). Biochemical, clinical, and ongoing pregnancy rates were similarly not affected by any of the three parties smoking habits at the time of ART treatment. Furthermore, the analysis of interactions between each party smoking status (passive smoking) did not yield statistically significant results.
Limitations, reasons for caution: Smoking habit at the time of treatment was self-reported, increasing the risk of under reporting.
Wider implications of the findings: Our results question the concept of tobacco-induced alteration of uterine receptiveness, and suggest that smoking habits at the time of the treatment might not be the most relevant parameter to evaluate in order to decipher the potential smoking induced alteration of oocyte quality. Length of exposure could be relevant.
Trial registration number: Not applicable.

P-391 Progestin-primed ovarian stimulation in combination with clomiphene citrate in normalovulatory women undergoing IVF/ICSI treatments: a prospective randomized controlled trial

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Study question: Whether Progestin-primed ovarian stimulation(PPOS) in combination with clomiphene citrate can reduce Gn dosage and how endocrine hormones change during Controlled ovarian hyperstimulation(COH).
Summary answer: Clomiphene citrate co-treatment with PPOS decrease gonadotropin dosage and improve LH level, without changing the number of oocytes retrieved and viable embryos compared with PPOS.
What is known already: Progesterone, whether exogenous applied in the follicular phases or endogenous generated in the luteal phases can effectively prevent premature LH surge. The Gn dosage and the duration of Gn administration are higher and LH concentrations are lower in the PPOS than in the short protocol. Clomiphene citrate has been proved effective in decreasing Gn dosage in GnRH-antagonist protocol, while no relevant investigations were reported in PPOS.
Study design, size, duration: A prospective randomized controlled trial was performed in 242 patients with normal ovarian reserve between June 2015 to October 2015. They were allocated into two groups by simple randomization: HMG+MPA+CC (group A, $n = 121$) or HMGMPA (group B, $n = 121$).
Participants/materials, setting, methods: In group A, clomiphene citrate (50 mg/d) in combination with hMG (150 IU) and MPA (10 mg/d) were applied simultaneously from cycle day 3. Equal starting doses of hMG and MPA were administered for group B. Ovulation was co-triggered by a GnRH agonist (0.1 mg) and hCG (1,000 IU) when dominant follicles matured and viable embryos were cryopreserved for later transfer in both protocols. The primary outcome measure for this study was the number of oocytes retrieved.
Main results and the role of chance: No statistically difference was found in the number of oocytes retrieved (9.1 ± 5.8 vs. 9.8 ± 8.1, $p > 0.05$) and viable embryos (3.6 ± 2.7 vs. 3.6 ± 3.4, $p > 0.05$) between the two groups. Group A received significantly lower Gn dose (1,349.4 ± 189.4 IU vs. 1,463.4 ± 364.6 IU, $p < 0.01$), and shorter Gn duration (8.9 ± 1.0 days vs. 9.3 ± 1.9 days, $p < 0.05$). In group A, LH value on the trigger day was higher than in group B (3.7 ± 1.8 IU/L vs. 1.6 ± 1.2 IU/L, $P < 0.01$). *P* value and E2 value on the trigger day were higher in group A than group B. Other parameters such as the number of mature oocyte, fertilization, cleavage were similar ($p > 0.05$). During the following up of FET, a total of 140 women completed 152 FET cycles, no significant difference was found in the clinical pregnancy rate (62.5% (45/72) vs. 63.8% (51/80), $p > 0.05$) and implantation rate (45.9% (61/133) vs. 47.9% (69/144), $p > 0.05$) between the two groups.
Limitations, reasons for caution: This study was for normal ovarian reserved women, more researches are still needed to testify whether it was applicable for different kinds of patients such as PCOS and DOR.
Wider implications of the findings: The co-treatment of clomiphene Citrate with PPOS can effectively prevent premature LH surge and decrease Gn dosage as well as its duration, without adversely affecting the pregnancy outcome. This protocol will be promising in combination with freeze-all policy.
Trial registration number: The trial was registered with the Chinese Clinical Trial Registry (ChiCTR-IPR-15006521)

P-392 The role of Clomiphene Citrate (CC) co-treatment with Progesterone-Primed Ovarian Stimulation (PPOS) protocol in subfertility women with PCOS undergoing IVF treatment: a randomized controlled trial

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Study question: What is the role of CC co-treatment with PPOS based on the freeze-all strategy during controlled ovarian stimulation (COH) in women with polycystic ovarian syndrome (PCOS)?
Summary answer: CC co-treatment with PPOS is effective to improve the LH level without LH surge, shorten the stimulation duration without adversely affecting the pregnancy outcome meanwhie.
What is known already: Our previous study indicated that progesterone could prevent premature LH surge in the luteal-phase ovarian stimulation and follicular-phase ovarian stimulation in PCOS patients. However, due to the persistent *P´* suppression to the pituitary, there is a decreasing trend in serum LH values during the simulation, it needs more Gn dose to stimulate the follicular development in the PPOS protocol compared with the short protocol. Therefore, we conducted a prospective study of CC co-treatment in the PPOS, to evaluate the endocrine characteristics and the clinical outcomes in PCOS patients undergoing IVF/ICSI treatment.
Study design, size, duration: In the prospective study, a total of 220 PCOS patients undergoing PPOS treatment were recruited between April 2014 and November 2015 and randomized allocated into two groups: hMG and MPA combined with CC (the study group, $n = 110$) or hMG and MPA (the control group, $n = 110$).
Participants/materials, setting, methods: In the study group, the initiating dose of hMG (150 IU) and MPA (10 mg/d)combined with CC (50 mg) were administered beginning on menstrual cycle day 3, while the starting dose of hMG (225 IU) and MPA (10 mg/d) were administered for the control group. The hMG dose could be adjusted depending on ovarian response. Ovulation was co-triggered by GnRH agonist (0.1 mg) and hCG (1,000 IU) when dominant follicles matured. Viable embryos were cryopreserved for later transfer in both protocols.
Main results and the role of chance: The study group was characterized by a shortened stimulation duration (9.14 ± 1.3 vs. 11.07 ± 1.24, $P < 0.05$) and lower stimulation dose of hMG (1,486.34 ± 360.36 IU vs.1,930 ± 377.72 IU, $P < 0.05$). The number of oocyte retrieved, MII oocytes, the fertilized oocytes and the cryopreserved embryos in the study group were lower than the control group ($P < 0.05$). The LH levels in the study group showed a slightly rise initially, then a downward trend, and average LH values on the trigger day was significantly higher than the control group (4.49 ± 2.49 vs. 2.52 ± 2.09 $P < 0.05$), moreover, no LH surge was found in the two groups. The pregnancy outcomes in FET cycles including implantation rate(51.09% vs. 45.04%), clinical pregnancy (72.86% vs. 62.09%)and ongoing pregnancy (62.86% vs. 54.8%) were comparable between the two groups, which indicated that the embryos originating from MPA and hMG co-treatment CC regimen had similar developmental potential. There were twenty three cases presenting LH levels higher than 10 IU/L in the two groups, and 16 out of 23 patients achieved ongoing pregnancy. The cycle cancellation rate due to no viable embryos was not different between the two groups (4.55% vs. 2.73%, $P > 0.05$). The incidence of OHSS was low between the two groups with no significant difference ($P > 0.05$).
Limitations, reasons for caution: The main limitation of this work is the absence of the live-birth outcomes following PPOS. Furthermore, questions for long-term safety of off-spring in PPOS remain.
Wider implications of the findings: This is the first trial to establish a novel regimen of CC in combination with PPOS in patients with PCOS undergoing IVF, Further studies will be performed to optimize and individualize the selection of the dose of progestin, clomiphene citrate and hMG initiating.
Trial registration number: ChiCTR-IIR-16007770

P-393 A randomized comparison of live birth rates following hormone replacement regimens for thawed blastocyst transfer

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Study question: Does the hormone replacement protocols used in thawed blastocyst transfer affect the live birth rate ?

Summary answer: Our data indicate the hormone replacement protocols produced significantly different live birth rates after thawed blastocyst transfer.
What is known already: There are no randomized controlled trials evaluating the live birth rates after thawed blastocyst transfers in patients treated with various hormone replacement regimens.
Study design, size, duration: A prospective randomized controlled trial was conducted in patients. We evaluated the live birth rates of two different hormone replacement regimens used for thawed blastocyst transfers. This study enrolled 300 women (median age 37.3 years) undergoing *In vitro* Fertilization (IVF) at our clinic in Japan from July 2014 to December 2015.
Participants/materials, setting, methods: The participants were randomized using a computer-generated randomization. There were two study groups; a Julina (®) group (n = 150; 4 mg/day) and an Estrana tape® group (n = 150; 0.72 mg, 4 pieces per 2 days).
Main results and the role of chance: On the 12th cycle day, the endometrium thickness was 8.86 ± 1.23 mm in the Julina ® group and 7.71 ± 0.23 mm (mean ± SD) in the Estrana tape® group. The endometrium thickness in the Julina® group was significantly higher than in the Estrana tape® group (P < 0.001). The serum estradiol (E2) levels were 279.86 ± 156.83 pg/ml in the Julina® group and 462.53 ± 296.0 pg/ml in the Estrana tape® group. The serum E2 levels in the Estrana tape® group were significantly higher than those in the Julina ® group (P < 0.001). The live birth rate in the Estrana tape® group was higher than that in the Julina® group (39.3% versus 28.0%, P = 0.038, odds ratio 1.67, 95% confidence interval: 1.03–2.70). The regimens were well-tolerated, and there was no difference in serious adverse events.
Limitations, reasons for caution: The conclusions of this study are limited to the two hormone replacement regimens examined in our patient population.
Wider implications of the findings: The serum E2 levels are correlated with differences in the live birth rate. The Estrana tape® hormone replacement protocol led to a higher live birth rate following the thawed blastocyst transfer procedures.
Trial registration number: UMIN000015488

P-394 Association of untreated subclinical hypothyroidism with live birth rates following *in vitro* fertilisation: a cohort study

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Study question: Is untreated subclinical hypothyroidism (SCH), defined as thyroid-stimulating hormone (TSH) values of 2.5–4 mIU/L in the presence of normal free thyroxine (FT4) levels, associated with live-birth rates (LBR) after *in vitro* fertilisation?
Summary answer: Untreated pre-cycle SCH, defined as TSH values of 2.5–4 mIU/L with normal FT4 levels, is not associated with LBR after *in vitro* fertilisation (IVF).
What is known already: Overt hypothyroidism has been implicated in poor reproductive, obstetric and foetal outcomes. However, the evidence surrounding the impact of SCH in the sub-fertile patient undergoing assisted reproductive technology (ART) remains controversial, particularly in the setting of moderately elevated TSH. Whilst some studies have suggested an increased prevalence of SCH in infertile women compared with the general female population, it remains unclear whether SCH adversely impact IVF success, thereby causing considerable debate on the need for treatment. Intriguingly, there have also been suggestions of a possible correlation between SCH and diminished ovarian reserve (DOR), however this too remains inconclusive.
Study design, size, duration: A retrospective analysis of IVF outcomes of untreated SCH patients compared to age-matched euthyroid controls. The primary end-point was LBR. Secondary end-point was clinical pregnancy rates (CPR). The antimüllerian hormone (AMH) test was used as surrogate measure for ovarian reserve. For a 10% difference in LBR, assuming two-tailed outcomes with ∝ minus error probability of 0.05 and power level of 0.8, a sample size of 203 is required for the study group.
Participants/materials, setting, methods: All fresh IVF cycles of women <38 years of age between 2011 and 2015 were retrieved from the IVFAustralia database. Only those patients who had underwent Thyroid Function Tests (TFTs) were included. Differences in proportions of categorical variables were evaluated with Chi-squared test. Unpaired Student's t-tests and single-factor analysis of variance were used to analyse continuous variables. The level of significance was set at two-tailed p-values of <0.05.
Main results and the role of chance: A total of 207 SCH patients were included in this study and were compared against 621 age-matched euthyroid controls, with a mean age of 34.1 years. The study group had a significantly elevated TSH compared to the control group (2.97 vs. 1.60, p < 0.01) with comparable FT4 levels (14.2 vs. 14.5, p = NS). The average number of oocytes retrieved (11.5 vs. 10.3) and fertilisation rates (59.7% vs. 60.0%) were similar in both groups. There was no significant difference in the LBR (27.1% vs. 25.4%) and CPR (30.0% vs. 28.8%) between the SCH and control groups (p = NS). No statistically significant difference was present in AMH levels between patients with and without SCH (27.6 vs. 31.1, p = NS).
Limitations, reasons for caution: Although the two groups were age-matched, the retrospective nature of the study might have allowed for the presence of other sources of bias. In addition, the total sample size of both cohorts may be inadequately powered to detect the difference observed in the above outcomes.
Wider implications of the findings: Our findings support current literature which recommends, in patients with marginally elevated TSH between 2.5–4 mIU/L, the option of monitoring TSH levels and treating when levels 4 mIU/L as alternative to commencing treatment to maintain TSH <2.5 mIU/L.
Trial registration number: Not applicable.

P-395 Gene expression differences in human pre-ovulatory granulosa cells derived from large and small follicles

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Study question: Is there a difference in the gene expression profile of human preovulatory granulosa cells derived from large and small follicles?
Summary answer: 44 genes were up-regulated while only 5 genes were down-regulated in granulosa cells from large follicles compared to those from small follicles.
What is known already: Human granulosa cells play crucial role in folliculogenesis, oocyte maturation and ovulation. Gene expression microarray analysis has been performed in order to find candidate genes responsible for the modulation of follicular development and oocyte quality. To our knowledge, there are no data concerning human preovulatory granulosa cells regarding the follicle size.
Study design, size, duration: The study population consisted of 32 women with tubal and/or male-factor infertility who underwent controlled ovarian stimulation for IVF/ICSI, for a period of one year. The follicles were categorized into small follicles with diameter <12 mm (from 16 women) and large follicles with diameter >18 mm (from 16 women) and granulosa cells were collected after their aspiration. Granulosa cell culture and RNA isolation was performed and further analyzed by microarrays and qRT-PCR.
Participants/materials, setting, methods: Granulosa cells originated from large and small follicles were cultured for 2 days, harvested and RNA extraction was performed. RNA quality and integrity was determined by Agilent 20,100 bioanalyzer and DNA contamination by RT-PCR. Equal

amounts (400 ng) of total RNA from each woman were pooled prior to microarray analysis (Affymetrix HG-U133 Plus 2.0). Q-RT PCR was performed to verify the results in the pooled RNA samples as well as in other individual samples.
Main results and the role of chance: Analysis of the microarray data demonstrated an up-regulation of 44 genes (3 fold-up) and a down-regulation of only 5 genes (3 fold-down) in granulosa cells from large pre-ovulatory follicles compared to those from small follicles. qRT-PCR was performed in the 5 down-regulated genes (SOX4, NPY1R, NCAM1, MLLT10, SLC27AC) as well as in ADAMS28, SSP1 and ITGAM up-regulated genes in the pooled RNA sample and in additional individual samples derived from several women. NCAM1 and SLC27A6 were found to be down-regulated in granulosa cells from large follicles. However, the different expression of SOX4, NPY1R and MLLT10 between large and small follicles was not confirmed. Our results provide a basis for the identification of genes involved in the oocyte maturation and ovulation during the IVF procedure.
Limitations, reasons for caution: In order to confirm the results, more samples need to be tested. Additionally, a bioinformatic analysis should be performed in order to analyse the transcriptome and define the genes implicated in specific pathways for further analysis.
Wider implications of the findings: The oocyte maturation process as well as the quality of the oocyte, mainly controlled by granulosa cells, play a key role in IVF-outcome. Therefore, the study of granulosa cell transcriptome could reveal new regulatory molecules and provide new tools that could be used to improve the quality of the oocyte.
Trial registration number: –

P-396 Effect of regular exercise on reproductive function of aged female mice

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Study question: Can regular exercise improve the expression of ovarian anigogenic factors, oocyte quality and fertility potential in aged female mice.
Summary answer: Regular exercise induced by illumination with incandescent lights in aged mice improves their reproductive outcomes by improving ovarian function and oocytes quality.
What is known already: Some studies have reported the relation between exercise and angiogenesis (Gustafsson et al., 2001; Prior et al., 2004). Previously it has been reported that a single bout of moderately intense treadmill running upregulates VEGF expression of skeletal muscle in rat (Breen EC et al., 1996) and upregulation of VEGF mRNA also occurs during exercise in humans in both normal healthy individuals (Gustafsson et al., 1999; Richardson et al., 1999) and patients with heart failure (Gustafsson et al., 2001).
Study design, size, duration: Forty female mice of 30–32 weeks were divided into the two groups. One group (n = 20) were induced to stimulate exercise (physical activity) by illuminating incandescent lights (60 Watts, 220 V), placed on the top of the cage, starting at 11:00 a.m. for 30 min daily. The other group (n = 20) were served as control without the induction of exercise.
Participants/materials, setting, methods: C57BL inbred mice were used and purchased from Korea Experimental Animal Center (Daegu, South Korea). Mice were maintained on a light-dark cycle, with light on at 5:00 AM and off at 7:00 PM, and with food and water available ad libitum.
Main results and the role of chance: The total number of pregnant mice was 15 (75%) in the exercise group, which was significantly higher than 5 (25%) in the control group ($P < 0.05$). The mean number of offspring was also significantly higher in the exercise group (9.2) than the control group (6.3) ($P < 0.05$). The mean number of one-cell embryos retrieved and blastocyst formation rate were 12.6 and 43.8% in the exercise group and 10.8 and 8.1% in the control group with a significant difference ($P < 0.05$). Ovarian VEGF and eNOS expression was increased, but ovarian apoptosis was decreased in the exercise group.
Limitations, reasons for caution: First, this study did not use a general exercise equipment, such as treadmill. Second, this study did not examine the effect of exercise on implantation because a lot of studies have shown that physical activity can affect implantation of endometrium.
Wider implications of the findings: This study did not clearly elucidate the mechanism that exercise treatment results to improve ovarian function and oocyte quality. However, this study showed the increased ovarian VEGF and eNOS expression and decreased ovarian apoptosis.
Trial registration number: None.

P-397 The role of three-dimensional transvaginal ultrasonography as a predictor of endometrial receptivity in *in vitro* fertilization

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Study question: Is the three-dimensional ultrasound (3D-US) with power doppler better than two-dimensional ultrasound (2D-US) to endometrium analysis of infertile women undergoing *in vitro* fertilization (IVF)?
Summary answer: Endometrial volume evaluated by 3D-US is a stronger predictive factor of pregnancy in IVF cycles with transfer of fresh embryos than 2D-US endometrial thickness.
What is known already: The endometrial receptivity is influenced by biological mechanisms, hormone secretion, proteins expression and a favorable endometrial milieu is extremely important for the success of IVF cycles, presenting a relative contribution of 31–64% for pregnancy. What determines such endometrial receptivity remains controversial and 2D-US has been routinely used in IVF clinical practice to evaluate the endometrial characteristics to embryo transfers. However, the endometrium is a three-dimensional, volumetric and non-planar structure, thus endometrial volume may be supposed to be more reliable than thickness to functional assessment, and it can be assessed by 3D-US in the IVF cycles.
Study design, size, duration: This prospective observational study included 123 women undergoing standard protocols for ovulation induction to fresh embryo transfers at a private Assisted Reproduction Center, between 2012 and 2015. Participants were split into two groups according with the clinical results of treatment: pregnancy and no-pregnancy. The variables were compared between groups and a multivariate logistic regression model was used to evaluate the association of ultrasound variables on the outcome of clinical pregnancy, adjusted for variables possibly involved.
Participants/materials, setting, methods: All patients underwent 2D-US to endometrium thickness measurements and 3D-US for assessment of endometrial volume and vascularization (VI, FI, VFI), using equipment ACCUVIX XQ (Medison, Seoul, Korea) at follicle final maturation trigger day. All 2D-US and 3D-US were performed by the same professional. The VOCAL® instrument was the imaging program used to calculate the volume and vasculature index in 12 outlines endometrial plans to cover 180°.
Main results and the role of chance: The demographic characteristics were similar between pregnancy and non-pregnancy groups (age: 33.8 ± 4.0 and 35.2 ± 4.7, p = 0.114; antral follicle count: 8.1 ± 3.8 and 7.2 ± 3.5, p = 0.223; FSH dose administered: 1,897 ± 663 and 2,105 ± 699, p = 0.119). However, pregnancy group had higher number of MII oocytes collected (8.2 ± 4.7 and 6.4 ± 3.5, p = 0.020) and good embryos transferred (1.8 ± 0.8 and 1.5 ± 0.9, p = 0.036). Pregnancy rate was 31.7%. The endometrial thickness was marginally higher in pregnancy than no-pregnancy group (9.9 ± 2.7 and 9.1 ± 2.2, p = 0.09), while the endometrial volume presented significantly higher in the patients who become pregnant (4.8 ± 2.1 and 3.7 ± 1.8, p = 0.003). The parameters of endometrial vascularization (VI, FI and VFI) were similar. The multiple logistic regression analysis showed the endometrial volume was a predictive factor of pregnancy (OR: 1.41, p = 0.003), wherein the endometrial volume increased the pregnancy rate in about 40% adjusted for confounders as age, antral follicle count, FSH dose, number of MII oocytes collected and good embryo transferred. The endometrial thickness was a weaker predictor of pregnancy (OR: 1.20, p = 0.05),

also adjusted for confounders. The ROC curve analysis showed that an endometrial volume of 2.0 cm^2 is a predictor of pregnancy ($p = 0.002$) with sensibility of 95% and specificity of 82%.
Limitations, reasons for caution: This is a prospective observational study evaluating the endometrial characteristics by 2D-US and 3D-US to estimate endometrial receptivity and it influence in conception. Ovarian stimulation response and embryo quality were not similar between pregnant and no-pregnant, but the multiple regression analysis were carried out to adjust for those confounders.
Wider implications of the findings: 3D-US is an accurate measurement of endometrial receptivity. In addition, it is non-invasive, relatively inexpensive, and, most importantly, has a high reproducibility intra and inter observer. Our findings support that 3D-US is a better predictor of pregnancy chance than 2D-US and can be used for routine in IVF cycles.
Trial registration number: Not applicable.

P-398 Ovarian stimulation in oocyte donors is associated with high expression of mRNA coding for apoptotic markers in cumulus cells

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Study question: Are there similar levels of apoptotic markers in cumulus (CC) and granulose cells (GC) of periovulatory follicles from fertile oocyte donors undergoing gonadotropic stimulation?
Summary answer: We found that ovarian stimulation to IVF in fertile women is associated with higher mRNA expression of apoptotic markers in cumulus cells, specifically caspase 3
What is known already: We have recently demonstrated that oxidative stress (OS) is involved in apoptosis of cumulus and granulose cells. The quality of a follicle and an oocyte has been estimated in terms of the incidence of apoptosis in granulosa cells in the patients involved in *in vitro* fertilization (IVF) treatment. On the other hand, we now know that GCs apoptosis is an important event in follicular atresia even though our understanding of the mechanisms that regulates GCs and CCs apoptosis is limited, and scarce knowledge exists about how CCs and GCs might be affected by ovarian stimulation in fertile women.
Study design, size, duration: This observational, prospective study compared the expression of mRNA coding for apoptotic markers in 185 oocyte-cumulus and 114 oocyte-granulose complexes retrieved from 18 healthy fertile oocyte donors <35 years, undergoing *in vitro* fertilization (IVF) during the period of June to November 2015. Written informed consent was obtained from the participants, and the local ethics committee approved the study. Only donors with at least a previous cycle with pregnancy were included.
Participants/materials, setting, methods: Donors were stimulated with the same protocol (FSHr and triggering with GnRH analogues). After collection of GC and CC cells from each donor, the samples were processed for mRNA levels of apoptotic markers: BcL-2, Bax, caspase-3 and caspase- 9 were assessed carrying out using real-time polymerase chain reaction (RT-PCR) analysis on the day of oocyte retrieval. The relative expressions of mRNAs of apoptotic markers in cumulus and granulose cells removed from individual oocytes were compared.
Main results and the role of chance: Results obtained from comparative RT-PCR analysis revealed that the mean relative levels of mRNA coding for caspase-3 were significantly increased ($p < 0.001$) in CCs (1.009 ± 0.12), from oocyte donors compared with CGs (0.343 ± 0.01) from the same women. The mean relative levels of mRNA coding for caspase-9 were also increased in CCs (1.05 ± 0.06), from oocyte donors compared with CGs (0.81 ± 0.06) although without statistically significant differences ($p = 0{,}069$).

No significant difference between groups was observed in mRNA coding for BcL-2 (CCs: 1.36 ± 0.05; CGs: 1.47 ± 0.02; $p = 0.39$), and BAX (CCs: 1.26 ± 0.06; CGs: 1.19 ± 0.01; $p = 0.139$)
Limitations, reasons for caution: Although it has been shown different levels of caspase 3 and 9 in CCs and GCs in fertile women undergoing ovarian stimulation to IVF, its implication in ovarian controlled hyper stimulation needs to be addressed in further studies on a larger sample of women.
Wider implications of the findings: These results indicate that CCs from fertile women subjected to ovarian stimulation, might suffer apoptosis in greater degree than CGs and are taken as an evidence for reduced defence against reactive oxygen species (ROS) in CCs during reproductive treatment.
Trial registration number: No clinical trial.

P-399 Expression of substance P, hemokinin-1 and the tachykinin NK1 receptor in human granulosa and cumulus cells

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Study question: Are substance P (SP), hemokinin-1 (HK-1) and NK1 receptor (NK1R) expressed in human ovarian cells?
Summary answer: SP, HK-1 and NK1R are expressed and act in coordination with kisspeptin in ovarian granulosa cells.
What is known already: The neurokinin B (NKB)/NK3 receptor (NK3R) and kisspeptin (KISS1)/kisspeptin receptor (KISS1R), two systems which are essential for reproduction, are expressed in human granulosa cells and contribute to the control of fertility by acting directly on the gonads. However, little is known about the presence and role of other members of the tachykinin family in human granulosa and cumulus cells.
Study design, size, duration: Samples were provided by 33 oocyte donors undergoing controlled ovarian stimulation at the clinic IVI Sevilla (Seville, Spain) between January 2012 and December 2013. Follicular fluid samples containing mural granulosa cells (MGCs) and cumulus-oocyte complexes (COCs) were collected after transvaginal ultrasound-guided oocyte retrieval. Cumulus cells (CCs) surrounding the oocyte were removed by using cutting needles, by subsequent treatment of COCs with Hyaluronidase and by carefully removing the CCs of the *corona radiata* with denudation pipettes.
Participants/materials, setting, methods: RT-PCR, quantitative real-time PCR, immunocytochemistry and western blot were used to investigate the pattern of expression of SP, HK-1 and NK1R mRNAs and proteins in MGCs and CCs. Intracellular free Ca^{2+} levels and $[Ca^{2+}]i$ in MGCs after exposure to SP or kisspeptin in the presence of SP, in the presence or not of tachykinin receptor antagonists, were also measured.
Main results and the role of chance: SP, HK-1 and NK1R were all expressed, at the mRNA and protein levels, in MGCs and CCs. Regarding NK1R, only the truncated isoform, NK1R-T, was detected. SP and HK-1 mRNA levels were similar in MGCs and CCs while NK1R-T mRNA was higher in MGCs, in comparison with CCs from the same donor. SP failed to induce any change in $[Ca^{2+}]i$ but reduced the $[Ca^{2+}]i$ increase produced by exposure to kisspeptin and the inhibitory effect of SP was suppressed in the presence of a cocktail of antagonists selective for the three known tachykinin receptors.
Limitations, reasons for caution: MGCs and CCs were obtained from oocyte donors undergoing ovarian stimulation which in comparison with natural cycles, may have affected gene and protein expression in granulosa cells.
Wider implications of the findings: Our data demonstrate that, in addition to NKB, other members of the tachykinin system are expressed and act coordinately with kisspeptin to regulate granulosa cell function in the human ovary.
Trial registration number: This is not a clinical trial.

P-400 Comprehensive analysis of body mass index effects on *in vitro* fertilization outcomes

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Study question: What is the effect of body mass index on retrieved oocytes number and oocytes quality among couples undergoing *In vitro* Fertilization in an Italian population?
Summary answer: Results show a significantly reduced percentage of mature oocytes when comparing obese (BMI ≥30 kg/m^2) and normal-weight patients (BMI = 18.50–24.99 kg/m^2).
What is known already: Conflicting evidence exists with regard to the effects of a raised body mass index on the outcome of ART. Even less clear is whether BMI acts with a potential detrimental effect on IVF outcomes via a deleterious effect on innate quality of oocytes or on the environmental milieu within the uterus.
Study design, size, duration: Data from 1,602 women (aged 20–45 years) treated at San Raffaele Hospital, a large university-affiliated infertility center, undergoing their first IVF/ICSI cycle, were analyzed retrospectively. Data were collected from January 2012 to December 2014.
Participants/materials, setting, methods: Patients were divided into four BMI categories. Baseline characteristics of the four BMI groups were compared with the use of Pearson chi-square test for variables categories and one-way analysis of variance for continuous variables, using Tukey's post hoc test for multiple comparisons. Outcomes of cycles were compared between BMI groups with the use of logistic regression to calculate the odds ratio, with their 95% confidential interval.
Main results and the role of chance: A significantly reduced percentage of mature oocytes was reported when comparing obese and normal-weight patients, (respectively 67.24 ± 30.65 and 76.9 ± 24.8, p = 0,005), resulting in a significant lower number of oocytes used for IVF procedures (in obese and normal-weight women respectively: 4.2 ± 3.4 and 6.0 ± 4.1, p = 0.003). Nevertheless, after adjusting for maternal age and other confounders, pregnancy rate was similar across different BMI categories. No statistically significant differences were observed neither for miscarriage nor for live birth rate. However a significant increased OR could be reported for miscarriage if all patients with BMI ≥25 were grouped.
Limitations, reasons for caution: Results are limited by the retrospective nature of the study; data have been obtained from a single institute. We considered only first ART cycles to minimize potential confounders by multiple failed cycles, but this might confer a potential limitation.
Wider implications of the findings: Appropriate counselling to encourage weight loss may help these patients but this strategy might be very time consuming. Number and quality of embryo are not severely affected by an high BMI while the maintenance of pregnancy seems to represent an important issue in these women.
Trial registration number: NA.

P-401 Ovarian rejuvenation and folliculogenesis reactivation in peri-menopausal women after autologous platelet-rich plasma treatment

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Study question: To explore whether autologous ovarian platelet-rich plasma (PRP) treatment could result in ovarian rejuvenation and subsequent folliculogenesis reactivation in peri-menopausal women.
Summary answer: Our data show, for the first time, the successful temporary ovarian activity restoration in peri-menopausal women after an autologous ovarian platelet-rich plasma treatment.
What is known already: PRP constitutes a concentrated source of growth factors and cytokines. Numerous studies in various medical fields have demonstrated the beneficial effects of PRP on tissue and angiogenesis regeneration. PRP treatment has been shown to improve vascularization and quality of an implant in an autologous ovarian transplantation, while when administered intrauterinely PRP has been shown to promote endometrial growth in cases with poor endometrial quality. The study of an ischemia/reperfusion injury rat model has shown that PRP treatment diminishes the oxidative stress and the ovarian histopathology caused by the bilateral adnexal torsion, while it increases the peritoneal vascular endothelial growth factor.
Study design, size, duration: Eight peri-menopausal women undergoing PRP treatment constituted the study population. All subjects, aged 45.13 ± 4.42 years, had absence of menstrual cycle for 4.88 ± 1.13 months. The FSH, LH, E_2 and AMH levels were determined before the PRP treatment and at monthly intervals after the PRP treatment in order to monitor the ovarian function. The presence of developing follicles was confirmed by ultrasound scan.
Participants/materials, setting, methods: PRP was prepared using the RegenACR®-C Kit and was infused into the ovaries using a transvaginal ultrasound-guided injection. All patients underwent natural cycle IVF without any ovarian stimulation or GnRH antagonist supplementation. When a follicle of >16 mm was observed, ovulation triggering was achieved with 5000 IU of hCG and follicle aspiration was performed 32 h later by the transvaginal route. The follicular size, the follicle and oocyte numbers were recorded during oocyte retrieval.
Main results and the role of chance: The successful ovarian rejuvenation was confirmed by the menstrual cycle restoration 1–3 months after the ovarian PRP treatment. The oocyte retrieval was successful in all cases, resulting in 2.50 ± 0.71 follicles of 15.20 ± 2.05 mm diameter, 1.50 ± 0.71 oocytes and 1.50 ± 0.71 MII oocytes. All mature oocytes were inseminated by ICSI and the 1.50 ± 0.71 resultant embryos were cryopreserved at 2pn stage until transfer. To date, no embryo transfer has been performed. Given the highly angiogenic ovarian structure and the critical role of various platelet derived factors for the vascular activation and stabilization, we could assume that PRP infusion probably enriched the dysfunctional, peri-menopausal ovarian tissue with the essential factors for angiogenesis and normal vascular function leading to tissue regeneration. As far as the observed folliculogenesis is concerned, many peri-menopausal women may maintain a restricted amount of inactive primordial follicles, that could be activated by the PRP growth factors or the subsequent ovarian tissue regeneration and maturate into preantral and antral follicles. Indeed, platelet-derived growth factors (PDGFs), regulating cell growth and division, have been shown to enhance blood vessel formation and growth. PDGFs have been localized in human oocytes and granulosa cells, while their receptors in granulosa cells, suggesting a potential association with primordial follicle activation.
Limitations, reasons for caution: Our investigation was based on eight women of advanced maternal age undergoing PRP treatment and subsequent natural cycle IVF during their transition to menopause. Further studies in larger population groups, analyzing both peri- and post-menopausal women are needed to verify our findings.
Wider implications of the findings: After the verification of our preliminary results in larger population groups, PRP could be used as a first line treatment for the ovarian regeneration and the folliculogenesis reactivation of peri-menopausal women. PRP therapy may extend the fertility potential of peri-menopausal women, rendering oocyte donation IVF cycles as an ultimate option.
Trial registration number: N/A

P-402 Profile of the patient with low oocyte yield: predictors of the oocyte recovery

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Study question: Could we predict the number of oocytes obtained the day of the oocyte pick-up (OPU) and the risk of a low yield?
Summary answer: Number of follicles higher than 12 mm the day of the induction seems to be the best single predictor of the number of oocyte retrieved.
What is known already: Serum estradiol levels and follicular number and size by ultrasound scan the day of the induction are commonly used to predict the number of retrieved oocytes. But there is variability in the number obtained and, in approximately 1 to 5% of the retrievals, no oocyte is found (EFS; empty follicle syndrome). This syndrome is sometimes related to a human error or wrong drug administration. Other times no abnormal treatment or response has been described even in recurrent cases.

Study design, size, duration: We analyzed retrospectively 24434 OPU cycles from patients and donors undergoing ovarian stimulation between January 2011 and December 2013 in four clinics (IVI Valencia, IVI Barcelona and IVI Sevilla).
Participants/materials, setting, methods: We analysed different stimulation and patient variables. Simple and multiple linear regressions were used.

Using the number of follicles equal or higher than 12 mm as the best predictor, we have compared 4 models of low oocyte yield retrievals. We define a low yield when less than 1, 1.25, 1.5 or 2 standard deviations from the average were obtained. Multiple logistic regressions were used to build and compare the models.
Main results and the role of chance: The best single predictor is the number of follicles equal or higher than 12 mm (R^2 = 88.9%, $p < 0.001$), followed by the number of follicle equal or higher than 10 mm (R^2=88.5%, $p < 0.001$). Estradiol levels is the other good predictor predictor (R^2=78.3%; $p < 0.001$). With a R^2 between 35% and 5% we found the clinic, induction method, age, etiology, stimulation method, basal FSH and progesterone at induction and with less than 5%: type of patient, total gonadotropin dose, duration of stimulation, use of contraceptives, body mass index (BMI), stimulation protocol and other endocrine levels. No combination of variables showed a relevant improvement over the use of the number of follicles as the single predictor.
Some variables are found significantly in the four models: induction type, days of stimulation and BMI. Some variables are only found in the less restrictive models: the clinic, oral contraceptive use, basal estradiol or stimulation protocol. Progesterone level only appears in the most restrictive model. The less restrictive models are more related to the local clinical management of the patients. The most restrictive model is the one that points to the intrinsic patient characteristics that correlates to the low oocyte yield.
Limitations, reasons for caution: This is a retrospective study and would be necessary to test this model in a prospective study and confirm these findings.
Wider implications of the findings: Though the number of follicles over 10 or 12 mm is enough to predict the number of retrieved oocytes, there is no good predictor of a low yield. Nevertheless, the risk of obtaining a low yield is related to the progesterone level at induction, age and ovarian reserve.
Trial registration number: Not applicable.

P-403 Apoptosis and pAKT levels in cumulus cells of patients undergoing *in vitro* fertilization program with specific polymorphisms of gonadotropins and their receptors: a case-control study

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Study question: Is there a difference in oocyte competence among patients with different gonadotrophin polymorphisms, after ovarian stimulation with r-FSH?
Summary answer: Higher DNA Fragmentation Index and cleaved caspase-3 related to lower level of pAKT has been observed in patients with specific gonadotrophin polymorphism (FSHR and LHB).
What is known already: In our experience, the DFI, the percentage of cleaved caspase-3 and the pAKT on cumulus cells can be used as molecular markers of oocyte competence; The polymorphic variant of LHB is characterized by an extra glycosylation signal into the β subunit. This molecular variation influences the pharmacokinetic properties of v-betaLH, showing an elevated bioactivity *in vitro*, but a significantly shorter half-life in circulation if compared with the wild type LH. These patients show sub-optimal ovarian response after pharmacological ovarian stimulation with r-FSH; We know that FSH receptor (FSHR) variants, Thr307/Asn680 and Ala307/Ser680, are involved in the response to ovarian stimulation.
Study design, size, duration: The aim of the study was to determine the apoptosis levels (in terms of percentage of DNA fragmentation and percentage of cleaved caspase-3) and the pAKT levels as potential markers of oocyte competence in the cumulus cells of individual patients with specific FSHR and LHB polymorphisms. It was a retrospective study. It was included 36 patients. The duration of the study was 12 months.
Participants/materials, setting, methods: Patients were normo-responder patients with a normal basic level of FSH (<12 IU/mL), body mass index <28, aged <38 years old, with a minimum of 3 MII oocytes collected. The patients were treated with a GnRH agonist and r-FSH. Cumulus cells were collected after treatment with a hyaluronidase solution. SNPs of FSHR and LHB gene will be amplified by PCR in different tubes, using different primers.
Main results and the role of chance: In this study population we found the following phenotypes:

- for FSHR: A/T – S/N (n = 18); A/A S/S (n = 6); T/T N/N (n = 12)
- for LHB: W/W I/I (n = 23); W/R I/T (n = 13)

A higher level of apoptosis in terms of DFI and amount of active protein caspase-3 ($p < 0.05$ vs. all other combinations) has been observed in patients with phenotype A/TS/N associated with W/RI/T (double heterozygous), correlating with an inverse proportion of survival factor pAKT. The analysis of clinical data between the double heterozygous and other combinations showed that there are no statistically significant differences in terms of total number of oocytes, oocytes in Metaphase I, zygotes, high quality embryos. No statistical difference was found also in the pregnancy rate (25% in double heterozygous compared to 30% in other combinations).
Limitations, reasons for caution: The main limitations could be the low number of patients with polymorphic variants among the cohort of patients included in the study.
Wider implications of the findings: We expect that patients with double polymorphism for LHB and FSHR will produce oocytes with a limited competence after ovarian stimulation with r-FSH. So, it will be possible to personalize the ovarian stimulation according to polymorphic condition of the individual patient.
Trial registration number: The trial is an observational study and no registration is needed.

P-404 Dual trigger with GnRH-a and hCG compared with a standard dose of hCG in patients with few follicles in IVF – pilot randomized case control study

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Study question: To evaluate if dual trigger with GnRh-a and hCG improves the outcome in patients with low number of preovulatory follicles, compared with hCG alone.
Summary answer: Dual trigger improved the outcomes regarding cancellation, fertilization and pregnancy rates, although the number of total and MII oocytes was similar.
What is known already: The combination of GnRH-a plus hCG for final oocyte maturation have been previously studied in normo and high responder patients in order to decrease the incidence of severe ovarian hyperstimulation syndrome and increase pregnancy rate. More recently it was reported the successful use of "dual trigger" in a patient showing repetitive immature oocytes and empty follicle syndrome.

Pregnancy and live-birth rates have been improved with dual trigger in normo-responder patients.

To our knowledge this is the first prospective study in order to investigate the effect of dual trigger in patients with low response to gonadotropin stimulation for IVF.
Study design, size, duration: This is a Randomized Case Control Study in which 96 patients with low response to controlled ovarian hyperstimulation undergoing IVF-ICSI were included from January to December 2015. Patients were randomized with sealed envelopes into two groups: Group 1 (Dual Trigger): received r-hCG 250 μg plus triptorelin 0.1 mg and Group 2 (control): received r-hCG 250 μg alone. The oocyte pick-up was performed 35–36 h after.

Demographic data, cycle characteristics of ovarian stimulation and final outcomes were studied.

Participants/materials, setting, methods: We analyzed 96 women undergoing IVF-ICSI in a GnrH antagonist protocol who presented ≤5 follicles ≥16 mm during transvaginal ultrasound performed the day of final trigger. All patients received a maximum dose of either recombinant or urinary gonadotropin of 300 UI/day. They were divided into two groups according to the triggering agent used: Group 1 (Dual Trigger): $n = 48$ patients and Group 2 (Control): $n = 48$ patients.

Main results and the role of chance: Both groups were demographically comparable in terms of age, FSH/LH, HAM, number of previous cycles, infertility cause, days of stimulation and total dose of gonadotropins used.

The total number of oocytes retrieved and maturity rate were similar in both groups. In Group 1 cancellation rate was statistically significantly lower, the fertilization rate was higher although it didn't reach statistical significance, the number of embryos cleaved was statistically significantly higher. PR per started cycle was higher, even though it didn't reach statistical significance.

	Dual Trigger	Control Group	*p* Value
OOCYTES RETRIEVED, No. (±SD)	3.34 (1.8)	2.75 (1.61)	NS
MATURITY RATE, % (±SD)	82 (24)	84 (24)	NS
FERTILIZATION RATE, % (±SD)	74 (25)	57 (35)	0.07
EMBRYOS TRANSFERRED per patient No. (±SD)	1.44 (0.85)	1.06 (0.9)	NS
EMBRYOS CLEAVED, No. (±SD)	2.96 (1.05)	1.7 (0.83)	0.01
GOOD QUALITY EMBRYOS, % (±SD)	82 (33)	84 (32)	NS
CANCELLATION RATE, % (*n*)	6.25 (3/48)	29 (14/48)	0.03
PR per started cycle, % (*n*)	29 (14/48)	10 (5/48)	0.058
PR per ET, % (*n*)	43 (13/30)	16 (5/31)	0.08

Limitations, reasons for caution: There was a tendency of better results regarding fertilization and pregnancy rates in dual trigger group but it wasn't statistically significant, possibly due to the small number of patients included. Currently synchronization between nuclear-cytoplasmatic maturation is impossible to be checked in live oocytes.

Wider implications of the findings: Dual trigger can improve outcome in patients with low response. The benefit may be related to more synchronic cytoplasmatic-nuclear maturation which could have decreased cancellation rate, increased the number of cleaved embryos and pregnancy rate. These promising results have to be confirmed by a larger number of patients.

Trial registration number: –

P-405 Effect of recipient's female age on clinical outcomes in oocyte donation cycles

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Study question: Does maternal recipient's age affect cumulative clinical outcomes in oocyte donation cycles with frozen oocytes?

Summary answer: A trend towards a reduction in cumulative positive betaHCG, clinical pregnancy and implantation rates was observed in the group of patients with higher maternal age

What is known already: In analyzing the outcomes of oocyte donation cycles, most attention has been focused on comparing results between fresh and vitrified oocytes, on the efficiency of vitrification protocols and on the effect of oocyte storage time/method on survival rate. Surely, the oocytes status is essential to the outcome of donation cycles, especially when frozen oocytes are concerned. However, successful implantation depends also on endometrial receptivity. It has been proposed that uterine age may play a role in endometrial response at a molecular level. However, existing reports on this issue are conflicting and many of them indicate no effect of uterine age.

Study design, size, duration: In Italy, egg donation was reintroduced with the last Constitutional Court sentence in April 2014. Vitrified oocytes were either imported from a foreign bank or obtained from our own bank. Oocytes were thawed and all inseminated by ICSI after 2 h incubation at 37°C, 5% O_2 and 6% CO_2. Retrospective analysis of cumulative pregnancy and implantation rates in our population is presented here. Data analysis was performed with Fisher's exact test. Data are expressed as mean ± SD.

Participants/materials, setting, methods: Our population was divided into 3 groups based on maternal age of the recipient at the time of transfer: group 1) $N = 17$, age 28–38 (35.4 ± 2.77); group 2) $N = 80$, age 39–44 (42.8 ± 1.55); group 3) $N = 49$, age 45–50 (47.0 ± 1.16). Patients were prepared with GnRH agonist and increasing doses of oestrogens. With advanced maternal age (>38) and/or amenorrhoea, additional treatment with oestrogens and progesterone was provided (days 1–7: 4 mg oestrogens; days 8–14: 6 mg oestrogens; days 15–21: 6 mg oestrogens+progesterone).

Main results and the role of chance: 976 oocytes were thawed (average/patient 6.1 ± 1.15); survival rate was 86.2% (841/976); donor's mean age was 27.1 ± 3.87; fertilization rate was 64.9% (546/841). Out of 162 cycles, 13 were cancelled for oocyte degeneration ($N = 2$) or null embryo development; 146 cycles underwent fresh ET whereas in 3 cycles embryos were all frozen. Out of 146 cycles with fresh transfer, in 52 (35.6%) no supernumerary embryos were frozen whereas in 94 cycles, 1–6 embryos were vitrified (average/cycle 2.2 ± 1.18). Cumulative positive betaHCG, clinical pregnancy and implantation rates on 173 transfer cycles (146 fresh, 27 frozen) were, respectively, 60.0% (12/20), 50.0% (10/20) and 30.6% (11/36) in group 1; 54.4% (49/90), 42.2% (38/90) and 30.0% (51/170) in group 2; 42.2% (27/64), 35.9% (23/64) and 24.3% (27/111) in group 3 (NS). This result was not significant probably for the low number of cycles. In our overall population the cumulative abortion rate per clinical pregnancy was 25.4% (18/71). This value could be underestimated since 35 pregnancies are still ongoing; 18 pregnancies gave birth to 20 healthy babies (delivered at 32–38 weeks of gestational age): 5 females and 15 males

Limitations, reasons for caution: Data presented here derive from cycles still ongoing and not all frozen embryos have been thawed, therefore it was not possible to estimate weather an average of 6.1 oocyte/cycle was adequate to give birth. Complete information on abortion rate in relation to maternal age is not yet available.

Wider implications of the findings: The success of oocyte donation relates not only to oocytes, donor's age, freezing and storing methods. An altered uterine receptivity with advanced age could imply a lower implantation or higher miscarriage. We hypothesize that the number of starting frozen oocyte needed in donation cycles should change according to maternal age.

Trial registration number: Not applicable

P-406 Long term outcomes in IVF: a systematic review and meta-analytical estimate of cumulative live birth and dropout rates

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Study question: Although constituting the main endpoint of IVF, few studies estimate Cumulative Live Birth Rate (CLBR) and dropout rate (DOR), or compare countries or centers.

Summary answer: Point estimates and credible 95% $C_r I$ of CLBR or DOR are 41.6% [24.3–75.6] and 49.5% [21.3, 63.8] respectively, both strongly influenced by patient mix, country and center performance.

What is known already: CLBR and DOR are difficult to estimate, mainly due to missing data and switches between centers. No systematic review or meta-analysis was conducted on this question. Important differences are suspected among countries, centers, initial patient's selection, rate of cancellation, used ART and freezing techniques, number of embryos per transfer, items selected for statistics, and a possible trend of center performance in time. Moreover, the definitions of CLBR and DOR, and the statistical

techniques are heterogeneous between the studies, making the comparison difficult.

Study design, size, duration: We conducted a systematic review and meta-analysis on literature findings. The main endpoint was long term IVF outcome. All studies published from 1995 to 2015 and reporting estimates of CLBR or DOR were included. For each study, we additionally collected the year of publication, country, number of centers, duration of follow-up, and inclusion conditions. For studies reporting subgroups, CLB and DOR were documented for each category of age, indication, and rank of attempts.

Participants/materials, setting, methods: A literature search of peer reviewed papers was conducted, using keywords as dropout, long term, IVF, CLBR, excluding abstracts. CLBR estimate was the main endpoint, CLBR dispersion estimated by the credibility interval (defined as containing 95% of the studies). Our primary analysis was a random Model, illustrated by a Forest plot and measurement of between study effects measured by I^2 statistics. Secondary analyses included meta-regressive models using age, publication year, type of study as meta-variates.

Main results and the role of chance: 34 studies were found during 1995–2015 period, over 15 countries. 9 were prospective studies, 17 were multicenter studies. The mean follow-up duration was 60 ± 33 months. The methodological quality index varied within [0.51, 0.87]. The median number of patients and cycles per study were 1001 [IQ: 550, 3011], and 2367 [1328, 5310]. The overall estimate and credible 95% C_rI of CLBR are 41.6% [24.3–75.6], this value was heterogeneous by country (SD = 0.036), and center within country (SD = 0.061). An inverse U-curve quadratic effect of age and a linear positive trend in time (0.008/year [0.002, 0.014], $p < 0.001$) were identified. The adjusted CLBR for the population of reference (30 years old, study in 2015) was 47.9% [37.5, 58.4]. The overall DOR was 49.5% 95% C_rI [21.3, 63.8], found very heterogeneous by country (SD = 0.083), and center within country (SD = 0.092). A strong increasing linear effect of age (0.019/year, [0.015; 0.023], $p < 0.001$) but no positive trend in time were identified. The adjusted DOR for the population of reference (30 years old, study in 2015) was 39.1% [22.1, 56.4].

Limitations, reasons for caution: Major endpoint in IVF, CLBR is poorly known and very seldom evaluated. Important differences were observed on definition of CLBR and dropout. In particular, for some studies, the possible switch of patients to other centers was not reported or considered as missing.

Wider implications of the findings: This first international meta-analytical estimate provides evidence of a positive trend of CLBR with time, but a strong heterogeneity of the performance among the centers. The DOR is even more heterogeneous among centers and does not decrease with time. Collaboration among centers is needed to optimize Center performance.

Trial registration number: NO Trial registration number

P-407 A randomized trial comparing the efficacy and safety of rFSH+rLH vs. rFSH alone and hMG to induce ovulation in cycles for low complexity techniques

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Study question: What is the efficacy and safety of rFSH+rLH stimulation scheme compared with rFSH alone and hMG in cycles for intrauterine insemination or programmed intercourse?

Summary answer: Stimulation with rFSH+rLH shown a significantly higher number of developing follicles with fewer stimulation days and similar safety parameters compared with hMG and rFSH alone

What is known already: Ovarian stimulation and programed intercourse are considered the initial treatment of anovulatory infertility and/or idiopathic. The most used drugs to induce ovulation are clomiphene citrate, recombinant follicle stimulating hormone (rFSH) and menotropins (hMG), reporting ovulation rates of about 80%; while pregnancy rates are up to 30%. Nonetheless, recombinant luteinizing hormone (rLH) supplementation has been used for treatment of high complexity patients with hypogonadism-hypogonadotropic, over 35 years or poor responders, which have reported improved pregnancy rates. This stimulation scheme also could benefit to younger patients undergoing low complexity; however, it is necessary to demonstrate the efficacy and safety of the treatment

Study design, size, duration: A randomized clinical trial, open label, comparing three schemes of ovulation induction. The sample size calculated included 152 cycles as followed: Group A, (rFSH+rLH) 51 cycles, Group B (rFSH) 53 cycles, Group C (hMG) 48 cycles; from January to December 2015, obtained by probabilistic sampling for intrauterine insemination or programmed intercourse

Participants/materials, setting, methods: Women aged ≤35 years attended at Reproductive Medicine Department, with unexplained infertility or anovulation. Patients were requested to have a BMI ≤30 kg/m^2, unilateral or bilateral tubal permeability, normal thyroid function, baseline serum FSH level ≤10 UI/l. They were randomized into 3 groups. Ultrasound and estradiol level until at least one follicle reaches to 18–23 mm, hCG triggering and intrauterine insemination or timed intercourse were performed

Main results and the role of chance: Demographic characteristics were similar in 3 groups. Baseline levels of FSH, LH, estradiol and TSH were comparable in all patients. The efficacy was significantly higher in the hMG and rFSH+rLH groups with percentages of response of 96% and 98% of ovulatory cycles respectively, compared with rFSH alone group with 85% ($p = 0.02$). The canceled cycles were more frequent with rFSH treatment ($p = 0.009$). It also was observed that rFSH+rLH treatment has a significantly higher number of follicles of 18–23 mm ($p = 0.01$), estradiol levels on day 10 ($p = 0.001$), fewer days of stimulation ($p = 0.010$) and fewer total units administered ($p \leq 0.001$) compared with the other treatments. hMG and rFSH+rLH treatments were significant in endometrial morphology ($p = 0.01$), the number of patients with hCG triggering ($p = 0.02$) and intrauterine insemination ($p = 0.004$). The pregnancy rate was higher but not significant in rFSH+rLH group ($p = 0.37$). There were no serious adverse events reported in any studied group

Limitations, reasons for caution: The development of more randomized trials in our population is necessary to obtain more clinical evidence and statistical significance. Also should be important to include a greater number of cycles in multicentre future trials

Wider implications of the findings: The rFSH+rLH stimulation scheme is an effective and safe treatment for infertile women in cycles for low complexity, that can be considered as first-line treatment for younger patients with anovulation or unexplained infertility, based on fewer days of doses and stimulation with similar rates compared to other schemes already used

Trial registration number: IMSS: R-2015-1905-10

P-408 Subcutaneous aqueous versus vaginal progesterone gel for luteal phase support in intrauterine insemination cycles: a pilot randomized, controlled trial

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Study question: Is pregnancy rate with subcutaneous progesterone similar to vaginal progesterone gel when used for luteal phase support in controlled ovarian stimulation (COH)-intrauterine insemination (IUI) cycles?

Summary answer: The clinical pregnancy rates are comparable between subcutaneous and vaginal gel progesterone, with a difference between groups consistent with the non-inferiority of subcutaneous formulation.

What is known already: Luteal phase support has been demonstrated to increase pregnancy rates among women undergoing IUI when receiving gonadotropins for ovulation induction. No significant differences in clinical pregnancy rates have been reported between vaginal progesterone gel and other vaginal or intramuscular progesterone products by authors, but no study have been conducted on the effect of the new subcutaneous formulation in women undergoing COH-IUI cycles.

Study design, size, duration: This open-label, prospective randomized, controlled, parallel-group, two-arm, non-inferiority pilot study was performed between December 2014 and January 2016. A total of 246 patients who underwent urinary follicle stimulating hormone (FSH) preparation (Fostimon)-stimulated

IUI cycles were prospectively randomized into 2 groups for luteal phase support. The study group (n = 120) received aqueous subcutaneous progesterone (Pleyris), and the control group (n = 126) received vaginal progesterone gel (Crinone 8) supplementation.

Participants/materials, setting, methods: In total, 246 women 18–38 years old, with either primary or secondary infertility for at least 1 years, body mass index between 19 and 30 kg/m^2, Day 2 serum FSH <15 IU/ml, normal serum prolactin level, normal uterine cavity on hysterosalpingography or hysteroscopy undergoing COH-IUI were randomized to an aqueous preparation of progesterone administered subcutaneously (25 mg daily) or vaginal progesterone gel (90 mg daily).

Main results and the role of chance: Using a PP analysis, which included all patients who received IUI (Pleyris =120; Crinone 8 =126), the ongoing pregnancy rate per cycle for subcutaneous versus vaginal progesterone was 12.7 versus 11.9%, with a difference between groups of −0.8% (95% CI −3.4, 2.8), consistent with the non-inferiority of subcutaneous progesterone for luteal phase support. In addition, rates of initial positive β-hCG (14.4% subcutaneous versus 14.0% vaginal) and clinical intrauterine pregnancy with fetal cardiac activity (13.8 versus 12.9%) were comparable. Both formulations were well tolerated, with no difference in serious adverse events. The result in tolerability of the drugs was measured using a satisfaction score ranging from 0 to 10 and the satisfaction among patients receiving subcutaneous was similar to vaginal gel (7.8 ± 3.1 subcutaneous versus 7.1 ± 2.9 vaginal; p = 0.126). By rotating the sites of injections with each dose (abdomen, arm, thigh, or buttocks) women randomized to Pleyris significantly reduced skin irritation and pain and increased their satisfaction with the new formulation. Possibility of self-administration, no need for special "confidence" with lower genital tract, no vaginal side effects, no interference with sexual activity and improvement of menstrual pain in women with endometriosis were also reported as advantages of subcutaneous progesterone administration by patients.

Limitations, reasons for caution: The conclusions are limited to the progesterone dosing regimen studied and duration of treatment for the patient population examined in this study. Data on live-birth rates are still ongoing and the study was not powered to detect differences in term of patients' satisfaction with drugs

Wider implications of the findings: Subcutaneous progesterone is a new option for luteal phase support in women undergoing assisted reproduction cycles (both IUI or IVF) who for are not confident with their genital tract or prefer not to use a vaginal preparation or who wish to avoid the side effects of vaginal routes of administration.

Trial registration number: NCT02316626.

P-409 The influence of initiation of progesterone supplementation in IVF-ET outcome-a prospective, randomized, controlled trial

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Study question: What is the optimal time for initiation of luteal phase support (LPS) in in-vitro fertilization and embryo transfer (IVF-ET) cycles

Summary answer: Initiation of progesterone supplementation on day +1 of oocyte retrieval did not decreased clinical pregnancy rate and implantation rate

What is known already: There is a consensus in the literature that LPS is necessary for IVF cycles. There has been significant debate regarding timing, dose, and routes of P administration. With regard to the timing of P initiation, it has been proposed that early P administration may be of benefit for ET by relaxing effect of P on the uterus. Conversely, ART cycles may be associated with advancement of the endometrium leading to embryo-to-endometrial asynchrony and implantation failure. Too early administration of P may further expand this asynchrony. In China most centers start P administration on the day of oocyte retrieval.

Study design, size, duration: This prospective randomized control study was conducted on 187 patients who underwent long-protocol treatment from 1 Oct 2014 to 31 Jan 2015 at our hospital.

Participants/materials, setting, methods: A computer-base randomization divided the recipients into two groups when HCG trigger. The first group (group A) started progesterone supplementation 1 day after oocyte retrieval; the second group (group B) started progesterone supplementation on the day of oocyte retrieval. Both of them receive embryo transfer 72 h to 120 h after oocyte retrieval.

Main results and the role of chance: Two groups were equal in COH protocol and causes of infertility. There were no significant differences with regard to the serum estrogen and progesterone level on the day of HCG trigger, oocyte retrieval and embryo transfer. The clinical pregnancy rate (group A 54.3% vs. group B 50.5%), implantation rate (group A 37.6% vs. group B 37.0%), and early pregnancy loss rate (group A 6.0% vs. group B 2.1%) were similar between the two groups.

Limitations, reasons for caution: In this paper we study the initiation time of i.m progesterone. It suggest that different routs of P administration may have influence on LPS initiation point. Addition randomized clinical trials are needed to better define P start time for luteal support for vaginal P, which is our future research direction.

Wider implications of the findings: We found that postpone the initiation of LPS to 1 day after oocyte retrieval would not result in insufficient of progesterone level on embryo transfer day and decrease the clinical pregnancy rate in ART. Besides that postpone the initiation of LPS would relieve patient's pains of intramuscular injection.

Trial registration number: Our study has registered in Chinese Clinical Trial Registry and the registration number is ChiCTR-IPR-14005293

P-410 A retrospective cohort study to investigate AMH-tailored optimisation of controlled ovarian stimulation in IVF cycles

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Study question: Which pituitary desensitisation regime and initial dose of gonadotropins should be used in controlled ovarian stimulation (COS) for patients with different anti-mullerian hormone (AMH) levels?

Summary answer: We did not find effect of increasing gonadotropin doseon oocyte yeild Consequently, it may not be possible to optimise dose and regime according to AMH.

What is known already: Tailoring COS protocols according to AMH is believed to be an effective strategy for optimising ovarian response, and has been widely advocated.

Study design, size, duration: Retrospective cohort study covering the period 1st October 2008 to 8th August 2012. 1851 treatment cycles undertaken by 1430 patients were included. The study was not subject to attrition, as cancelled cycles were included in the analysis.

Participants/materials, setting, methods: Women of 21–43 years of age undergoing ovarian stimulation for IVF (possibly with ICSI) using their own eggs at the Reproductive Medicine Department of St Mary's Hospital. Regime (GnRH long agonist or GnRH antagonist) and initial gonadotropin dose was selected on the basis of AMH. Patients received doses of 75–150 IU (n = 297), 187–250 IU (n = 484), 300 IU (n = 919), 375 IU (n = 62) or 450 IU (n = 89).

Main results and the role of chance: Compared to 75–150 IU in a GnRH long agonist cycle, yield ratios (95% CIs) corresponding to number of oocytes retrieved per cycle were, in GnRH antagonist cycles: 0.76 (0.67 to 0.86) for 75–150 IU, 1.08 (0.90 to 1.30) for 187–250 IU, 1.04 (0.91 to 1.18) for 300 IU, 1.11 (0.90 to 1.37) for 375 IU and 0.94 (0.76 to 1.17) for 450 IU. In GnRH long agonist cycles, adjusted rate ratios (95% CIs) were 1.12 (1.01 to 1.25) for 187–250 IU, 1.17 (1.03 to 1.33) for 300 IU, 1.18 (0.92 to 1.51) for 375 IU and 1.07 (0.87 to 1.33) for 450 IU. There was no evidence to suggest that dose effects varied according to AMH (p = 0.60).

Limitations, reasons for caution: This was a retrospective, observational study where COS treatment was selected on the basis of AMH. Although we have adjusted for a large number of plausible confounders, confounding due to unmeasured factors remains a concern. The possibility of a dose-response was not completely ruled out by 95% CIs.

Wider implications of the findings: If there is no effect of increasing dose on stimulation response, then optimisation of COS according to AMH may not be feasible. Existing evidence for AMH-tailored COS has limitations, and an AMH-stratified RCT using clinical doses of gonadotropin in different pituitary desensitisation regimes is needed.
Trial registration number: Non applicable.

P-411 How low can you go? – cutoff limit for IUI in mill/ml

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Study question: How low is the cutoff concentration of motile semen in IUI to get a pregnancy rate of 5%?
Summary answer: Between the age of 17–39 a clear cutoff at 2 mill/ml motile semen cells were needed to achieve a minimum pregnancy rate of 5%.
What is known already: IUI is widely used in couples with unexplained infertility or mild to moderate male infertility. Despite the extensive literature in the area, the effectiveness of the IUI is still questioned.

IUI is known to increase the gamete density at the site of fertilization and thereby increase the pregnancy rate. Through literature, no consensus of the minimum concentration of motile semen, needed to achieve a pregnancy rate of 5%, is found. The correlation between the age of the women inseminated, the semen concentration and the pregnancy rate is also yet to be defined.
Study design, size, duration: In a retrospective study from 2012–2015, data of 4.322 IUI cycles was consecutively collected in a private clinical setting (Copenhagen Fertility Center, Denmark).
Participants/materials, setting, methods: All IUI cycles with use of homologous semen were included. The mean age of the women were 35 years. The mean motile semen concentration after washing was 30 mill/ml. All women were grouped into age brackets of: 17–29, 30–34, 35–39, >40 years of age. All inseminations were grouped according to motile sperm concentration count after washing: <2, 2–7.9, 8–49.9 and >50 mill/ml.
Main results and the role of chance: In all age groups, a pregnancy rate of less than 5% was seen when inseminated with <2 mill/ml. In the 17–29 bracket group a significant increase in pregnancy rate of 23% was seen when inseminated with >50 mill/ml compared to the women inseminated with <2 mill/ml (23% increase in pregnancy rate) 2–7.9 and 8–49.9 mill/ml ($p < 0.01$). In the 30–34 bracket a significant increase in pregnancy rate of 15% was seen comparing women inseminated with <2 mill/ml to women inseminated with >50 mill/ml ($p < 0.01$). Among the 35–39 bracket a significant increase in pregnancy rate of 13% was seen comparing women inseminated with <2 mill/ml with women inseminated with >50 mill/ml ($p < 0.05$). In the >40 bracket no pregnancies was found when inseminated with <2 mill/ml. No significant increase in pregnancy rate was seen when comparing the different semen concentration groups. The respective pregnancy rates was according to the semen concentration: 2–7.9 mill/ml = 6%, 8–49.9 mill/ml = 6% and >50 mill/ml = 5%.
Limitations, reasons for caution: This study includes only patients attending a private fertility clinic in Denmark, situated in Copenhagen. Therefore, the patients are selected from their geographic location. The next study in line could include patients outside the capital**.**
Wider implications of the findings: This large population study demonstrates the importance of a clear cutoff at 2 mill/ml for IUI treatment to achieve a minimum pregnancy rate of 5%. The low pregnancy rates in IUI treatment among women aged >40 underlines the importance of sufficient stimulation and fast conversion to IVF when needed.
Trial registration number: N/A

P-412 New findings on acute immunomodulatory changes during controlled-ovarian-stimulation

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Study question: May estradiol raise exert an immunomodulatory effect in women undergoing COS?
Summary answer: The acute increase of E2 during COS for infertility treatment does not seem to have a major impact on the immune system.
What is known already: The higher incidence of B-cell mediated autoimmune diseases in women of childbearing age have been imputed to the oestrogens power to promote a shift from Th1 to Th2 immune response. At this regard, experimental murine studies have recently demonstrated that the upregulation of E2 (17β-Estradiol) is capable to influence B cell development, selection, and activation, thus leading to a loss of B cell tolerance, and the promotion of B cell autoreactivity, lymphoproliferation, hypergammaglobulinemia, and autoimmunity.
Study design, size, duration: We conducted a prospective study on idiopathic infertile women aged between 18–43 years, scheduled for fresh non-donor IVF treatment between January 2011 and March 2014. All patients were defined normo-responders according to biochemical and sonografical features (Group_A: treatment group). We also recruited a cohort of normo-ovulatory age-matched healthy women (Group_B: control group) who spontaneously adhered to the aim of the study. Their serum samples were used for time-matched comparison with Group_A.
Participants/materials, setting, methods: In the setting of the Infertility Center of University of Padua, we enrolled 63 infertile normo-responder women (Group_A) undergoing COS and 39 normo-ovulatory healthy women (Group_B). We analyze for all patients serum levels of E2 and BAFF (B cell-activating factor), BAFF/E2 ratio, circulating IgM, IgG and IgA, ANA (anti-nuclear antibodies) and peripheral B-cell phenotype according the subsequent timing: T0 (hypothalamic-suppression), T1 (ovulation-induction) and T2 (βhCG-test) in Group_A; T0 (2nd day of spontaneous menstrual cycle), T1 (14th day) and T2 (21th day) in Group_B.
Main results and the role of chance: At T0, the comparison between women in Group_A versus Group_B showed no significant differences in terms of absolute value of E2 as well as absolute value of BAFF. Also considering the BAFF/E2 ratio, no significant difference was observed between Group_A and Group_B. At T1, a significant difference in absolute values of E2 between Group_A and Group_B was recorded ($p < 0.0001$), without any difference in terms of absolute value of BAFF and the BAFF/E2 ratio. Of the 63 women in Group_A, 15 became pregnant. Concerning the absolute values of BAFF, the comparison between the non-pregnant women of Group_A and Group_B showed no differences as well as the comparison between the non-pregnant versus the pregnant women of Group_A. Considering only Group_A patients, a correlation was observed between BAFF levels at T0 and IgM at T0 (rho= 0.401; $p = 0.009$), T1 (rho=0.496; $p = 0.002$) and at T2 (rho= 0.406; $p = 0.061$). Concerning the peripheral B cell subpopulation status in Group_A, a tendency was observed toward an expansion of the mature marginal zone CD19+ CD27+ IgD+ of memory B cells at T1 in comparison with T0 ($p = 0.083$), whereas the proportion of immature B-transitional-type 2 B cells CD19+ CD27 − IgDhiCD21hi was lower at T1 than at T0 ($p = 0.074$).
Limitations, reasons for caution: The small sample size, the fact that all infertile patients were affected by unexplained infertility, the relatively low pregnancy rate, the absence of evidences regarding the effects of different COS protocols, and the lack of data regarding pregnancy after spontaneous cycle represent potential bias of our study.
Wider implications of the findings: If our results will be confirmed, COS may be considered as a medical procedure with safe short-term effects on women immunological profile.
Trial registration number: N/A

P-413 Intra uterine insemination pregnancy outcome is not affected by a longer time interval between semen processing and insemination

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Study question: Is the pregnancy outcome of intrauterine insemination (IUI) affected by a longer time interval between semen processing and insemination?
Summary answer: There is no difference in on-going pregnancy rate between insemination directly after semen processing compared to insemination 24 h after semen processing.

What is known already: IUI outcome depends on multiple factors such as patient characteristics, timing of ovulation and semen-quality. Semen processing is essential since spermatozoa have limited survival capacity and other constituents in semen, such as leucocytes and dead spermatozoa, produce oxygen radicals that negatively influence the ability to fertilize the egg. Previous data suggest that IUI pregnancy outcome is enhanced by a short time interval between semen collection and semen processing before IUI. It has also been suggested that pregnancy outcome after IUI is enhanced by shortening the time interval between processed sperm and IUI, but the evidence for this is lacking.
Study design, size, duration: Historically in our clinic, if ovulation takes place on a Saturday, sperm is collected and processed on the Friday before. Several unpublished analyses in the past showed no negative effect on pregnancy outcome but a systematic evaluation of this policy was never performed. This is a single-centre, retrospective cohort study including 2154 IUI cycles in 1135 couples. To avoid duplicates we analysed only the last IUI. Data were collected between November 2005 and April 2015.
Participants/materials, setting, methods: patients in whom ovulation and insemination took place on Saturdays and semen was collected and processed on Friday for logistical reasons (delayed insemination) were compared to patients where processing of sperm and insemination took place on Friday (immediate insemination). Depending on the subfertility diagnosis, IUI-cycles were either with or without controlled ovarian hyperstimulation. Baseline characteristics and cycle specific information were collected. For statistical analysis independent T-test and Chi-square contingency test were performed.
Main results and the role of chance: In total 1135 couples were analysed. In 13% of the couples pregnancy occurred after immediate insemination (77/588) and 14% of the couples were pregnant after delayed insemination (77/547). Both groups had similar clinical characteristics, with all P values >0.05. After adjustment for female age, male age, type of subfertility, indication of IUI, type of stimulation and total motile count, there was no difference in on-going pregnancy rate after immediate or delayed insemination, odds ratio 0.89 (95% CI: 0.63 to 1.25).
Limitations, reasons for caution: This is a single centre, retrospective cohort study. Therefore, the level of evidence is moderate. We might have overestimated the overall pregnancy rate since we only evaluated the last IUI cycle of every couple.
Wider implications of the findings: This study suggests that there is no negative effect on on-going pregnancy rates when IUI with processed sperm is delayed. This approach allows additional flexibility for couples if the partner is not available on the day of ovulation. Also, semen processing on Fridays reduces the laboratory workload in the week-end.
Trial registration number: WO 15.111

P-414 "Operative complications and results after laparoscopic removal and transplantation of ovarian tissue: own experience in comparison with the literature"

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Study question: What is the frequency of surgical complications and the pregnancy rate occurring in laparoscopic removal and transplantation of ovarian tissue for fertility preservation in cancer?
Summary answer: Laparoscopic removal and subsequent transplantation of ovarian tissue is a procedure with a very low complication rate and a chance for pregnancy of about 15–30%.
What is known already: Fertility preserving techniques in oncological treatments are gaining an increasing weight. Long-term survival in cancer could have been prolonged and the fulfilling of the wish for a child is being shifted in later periods of life. At the same time, the gonadotoxic potential of oncological therapy did not decrease. A fertility-preserving option in oncological patients becoming more and more popular is the laparoscopic removal of ovarian tissue before and its transplantation after treatment. Until today, operative complications and pregnancy rates within this procedure have not been investigated very well.
Study design, size, duration: We performed a selective research of the literature yet being published in MEDLINE about operative complications and pregnancies in the context of removal and transplantation of ovarian tissue to preserve fertility. Altogether 16 publications about the removal and 15 publications about the transplantation of ovarian tissue were detected.
Participants/materials, setting, methods: Afterwards, we compared the results retrospectively with those of 29 patients treated at our centre between May 2007 and November 2014. So we combined the systematic review of the published literature with a retrospective analysis of operative and pregnancy outcome during the procedure of laparoscopic removal and transplantation of ovarian tissue.
Main results and the role of chance: Laparoscopic removal and transplantation of ovarian tissue are operative procedures with a low risk for complications. Potential risks such as post-operative haemorrhage or wound infection occur with a frequency of <1%. In most cases there is no complication at all. More than 60 children already have been born after removal and transplantation of ovarian tissue. This number is raising. In the 29 patients treated at our centre we did not observe any operative complication. There were 9 pregnancies with 5 live births in 7 different patients. The pregnancy rate of 24% at our centre is comparable with the frequency of pregnancies reported in literature of about 15–30%.
Limitations, reasons for caution: The evidence about complication rates of removal and transplantation found in literature is very limited. There are no prospective randomized controlled trials evaluating this field at all. The patient collective of our single centre is small and we performed a retrospective analysis.
Wider implications of the findings: In our eyes-due to the results-laparoscopic removal and transplantation of ovarian tissue should be regarded as a standard surgical procedure as any other laparoscopy else and it should be offered to every premenopausal woman with cancer and potential future wish for a child undergoing oncologic therapy.
Trial registration number: none

P-415 Initial trial of a serum diagnostic profile to predict outcome of embryo transfer

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Study question: Can a serum diagnostic profile at hCG+2 predict the outcome of a same cycle embryo transfer?
Summary answer: A serum biomarker panel shows promise as a same cycle prognostic test for embryo transfer outcome.
What is known already: Successful IVF requires both a quality embryo and a receptive endometrium. Currently there is no diagnostic test available to assess the quality of the maternal endometrium during an embryo transfer cycle. Previous studies focussed on gene or protein profiling of tissue and uterine secretions during the anticipated receptive period. However these approaches have limited utility within an embryo transfer cycle, due to tissue damage potentially compromising implantation and time constraints of sample collection and assay prior to embryo transfer. A serum assay performed at oocyte retrieval would overcome these limitations, informing of likelihood of successful transfer, and increase success rates.
Study design, size, duration: Serum was collected with informed consent at the time of oocyte retrieval from 397 women over an 18 month period. Of these women 280 (aged 23–46 years) underwent embryo transfer in the same cycle and are included in this analysis. Logistic modelling was performed and performance assessed by ROC analysis.
Participants/materials, setting, methods: Serum was assayed for Colony Stimulating factor-3 (CSF3), Interleukin-8 (IL8), Interleukin-6 (IL6), vascular endothelial growth factor (VEGF) and Interleukin-17A (IL17A) using Milliplex bead-based ELISA. C-reactive protein (CRP) was determined using Randox high sensitivity assay. Additional parameters, e.g., peak estradiol, progesterone, embryo quality, age, BMI, etiology, endometrial thickness, etc., were collected. Outcome of embryo transfer was determined as a confirmed pregnancy by ultrasound; biochemical pregnancies, Any not confirmed by ultrasound were excluded.
Main results and the role of chance: Logistic models combining the six biomarkers along with other parameters showed significance in predicting the outcome of embryo transfer for the women with confirmed pregnancy or no pregnancy outcomes. Four markers (CSF3, IL17A, VEGF and IL8) showed strong discrimination of pregnancy and no pregnancy outcomes, as did a number of ratio combinations of the six.

In logistic regression models, criterion values in ROC analysis were assigned to ensure prediction of successful pregnancies was as close to 100% as possible, at the expense of poorer detection of failed pregnancy.

For day 2/3 transfers (n = 100) ROC curve analysis gave AUC = 1.000 with significance $p < 0.0001$, thus 100% correct predictions, PPV of 100 and NPV of 100 were achieved. The major factors used in the analysis were, age, Il6, CRP, IL8, P4, E2, and endometrial thickness.

For day 5/6 transfers (n = 162) ROC analysis gave AUC = 0.873 with significance $p < 0.0001$, with a sensitivity of 97.8 and specificity of 47.0, NPV = 98.2 and PPV = 41.5.

The major parameters used were; age, IL6, IL8, VEGF, CRP, CSF3, IL17A, P4, E2.

Limitations, reasons for caution: This is a single site preliminary trial of a new diagnostic for receptivity. Numbers are limited and a second test data set to evaluate the true diagnostic performance is required.

Wider implications of the findings: The use of serum as opposed to invasive uterine tissue or fluid is negated. Potential identification of further biomarkers including those to identify specific risk factors, best therapy options and combination with embryo quality testing may provide a path to cycle monitoring and individualised therapy.

Trial registration number: Not applicable

P-416 A new approach for egg collection, using a thin egg-collecting needle in a natural cycle, that produces multiple mature eggs from each follicle

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Study question: We collected eggs from every follicle produced during a natural cycle, including the dominant follicle, and examined whether multiple mature eggs could be produced.

Summary answer: Overall, 2 mature eggs were obtained from 37% of natural cycles. The eggs collected demonstrated high developmental potency and fertility.

What is known already: Although previous reports indicates that multiple mature eggs can be obtained from every follicle, including small ones, in a letrozole stimulated cycle, there are few reports on egg collection from every follicle as part of a natural cycle.

Study design, size, duration: We collected eggs during a natural cycle from 247 women (age: 37 years or younger, treatment cycle: 251) between January 2014 and April 2015.

Participants/materials, setting, methods: We collected eggs from every follicle during a natural cycle (down to those measuring 5 mm in diameter). Thirty-four hours after treatment with a gonadotropin releasing hormone agonist nasal spray the dominant follicle reached 17 mm or more in diameter. Puncturing of small follicles was carried out using a thin egg-collecting needle (KITAZATO, 22 or 23 G). We performed single embryo transfer and, in cases in which the embryo remained, offered blastocyst culture.

Main results and the role of chance: The average number of punctured follicles was 4.8. The total number of mature eggs obtained from follicles was 372 (1.5-egg per cycle). At least 1 mature egg was collected after 205 cycles (82%), and 2 mature eggs or more were obtained after 92 cycles (37%). The maximum number of mature eggs was 8. Single embryo transfer pregnancy rates were 42% (66/158). Blastocyst development rate of the remaining embryos was 32% (32/100). Blastocysts were vitrified. Women who failed embryo transfer underwent freeze-thaw blastocyst transfer during the next cycle after the failed transfer. Their pregnancy rate was 46% (6/13).

Limitations, reasons for caution: Not available.

Wider implications of the findings: Egg collection during a natural cycle has no risk of ovarian hyperstimulation syndrome and is less stressful for patients because they require less medication. Although a thin needle is used, multiple mature eggs can potentially be obtained from every follicle. Pregnancy rates increased when using the remaining embryos.

Trial registration number: Not available.

P-417 When is enough enough? Pregnancy rate and cost-effectiveness of three cycles homologous intrauterine insemination (IUI) vs. one cycle mild ovarian stimulation *in vitro* fertilization (IVF)

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Study question: Is the pregnancy rate by one mild ovarian stimulation IVF cycle higher than by three IUI cycles?

Summary answer: Mild ovarian stimulation for IVF provides a higher pregnancy rate as well as being cost effective when compared to three IUI cycles

What is known already: IUI is a widely used treatment because of the low costs and minimal risks. IUI is often repeated more than three times before converting to IVF. In IUI, cycles with Clomiphene Citrate stimulation, the pregnancy rate is 10–15% per cycles in women under the age of 35, with open tubes and a normal semen sample. After three IUI cycles the pregnancy rate drops to 5%. In contrast, mild ovarian stimulation for IVF has a pregnancy rate of 35–37% pr cycle. It's cost-effective and patient-friendly regimens optimize the balance of outcome and risks of treatment

Study design, size, duration: In a retrospective study data from 2010–2015, in a Danish private fertility clinic, was collected. In all 897 IUI, cycles and 53 IVF cycles were included. Only IUI cycles with homologous insemination and only IVF cycles with mild ovarian stimulation were included. Pregnancy rate was measured as positive hCG per cycle

Participants/materials, setting, methods: In the IUI group the mean age was 33. All were treated for three cycles with 50–100 IU Clomiphene Citrate, low dose follicle stimulating hormone (FSH) or no treatment. In the IVF group, the mean age was 34. They were treated with either 20–40 mg Tamoxifen or 50–100 IU Clomiphene Citrate daily, supplemented with FSH 150 IU injections every other day. Transfer of only one good quality embryo was performed between days 2–3 from aspiration

Main results and the role of chance: In the IUI group of 897, 109 of the women had a positive hCG after the third IUI cycle. In the group of mild ovarian stimulation for IVF, 16 of the 53 women had a positive hCG. This showed a significant difference in positive hCG between the IUI group and the IVF group (12% vs. 30%; $P < 0.001$). There was no significant difference in patients' age group

Limitations, reasons for caution: The number of patients in the IVF group was 53. In a future study, a larger group of patients should be included

Wider implications of the findings: This study shows how the pregnancy rate of one mild ovarian stimulation for IVF (30%) is more than the double as effective as three cycles homologous IUI insemination (12%). This underlines the importance of a discussion of a faster conversion in treatment from IUI to IVF

Trial registration number: N/A

P-418 Evaluation of dose depended protective effect of resveratrol at cisplatin induced ovarian damage

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Study question: Does Resveratrol have protective effect against cisplatin induced ovarian damage and is this effect dose depended.

Summary answer: Resveratrol has a protective effect against chemotherapy induced ovarian damage and this protective effect strengthens with the increasing dosages of Resveratrol

What is known already: Cisplatin is one of the most effective chemotherapy agent for several kinds of solid organ cancers. However it has adverse effects on reproductive capacity and ovarian functions. Many researches were carried out to restorate these side effects of cisplatin and prevent ovarian functions in women at reproductive ages with cancer. Resveratrol is a polyphenol found in grapes and red wine and has antioxidant, anticancer, antiangiogenic and anti-inflammatory activity. Recently it was shown that resveratrol has a role

at chemosensitizing and protective at cisplatin induced nephrotoxicity and ototoxicity.

Study design, size, duration: This study was conducted in a University Surgical Research Center. Twenty four Sprague-Dawley rats were randomly divided into four groups. Group 1, 2 and 3 were injected 5 mg/kg cisplatin for 7 days, additionally group 2 was administered 10 mg/kg and group 3 was administered 100 mg/kg Resveratrol 24 h before and during cisplatin injection period. Group 4 was vehicle. Rats were sacrificed at the end of the experiment and their ovarian tissue were excised.

Participants/materials, setting, methods: Ovarian damage was evaluated by vascular congestion, edema, follicular degeneration and vacuolization at hematoxylin and eosin staining and apoptosis was evaluated immunohistochemically with anti-Caspase 3 antibody.

Main results and the role of chance: Vascular congestion, edema, follicular degeneration and vacuolization were severe and significantly increased in Cisplatin group ($p < 0.001$). The protective effect of Resveratrol was observed in both low and high dosages ($p = 0.006$ and 0.001 respectively) but the ameliorative affect was most striking in high dosage group ($p < 0.005$) as decreased congestion, edema, vacuolization and follicular degeneration. Anti-Caspase 3 staining was significantly higher and prominent in group 1 ($p = 0.031$). There were significantly lower anti-Caspase 3 staining in group 2 and 3 in comparison to group 1 ($p = 0.048$ for each group).

Limitations, reasons for caution: Confirmation of the results with the human tissues would be required in the future.

Wider implications of the findings: Our results are proved that Resveratrol is a potential agent for ovary preservation from chemotherapy induced toxicity and the protective affect is enhanced dose depended.

Trial registration number: Project number: 2015.106.02.3.

P-419 Individual fertility assessment and counselling predicts prolonged time to pregnancy – a prospective two year follow-up study of 519 women

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Study question: What is the predictive value of individual fertility assessment and counselling in terms of subsequent time to pregnancy?

Summary answer: The chance of achieving a spontaneous pregnancy within 12 months was reduced in women with at least one high risk score (OR 0.25, 95% CI 0.12–0.52).

What is known already: The reproductive patterns have changed substantially in the past decades. Several factors has led to increased demand for pro-fertility consultations: 1) postponement of family formation due to increased female education, 2) the option of single motherhood by donor insemination, 3) introduction of oocyte vitrification for non-medical reasons, and 4) the possible concealment of long-term oral contraceptive use on the ovarian reserve (Birch Petersen et al., 2015). The Fertility Assessment and Counselling Clinic (FACC) was initiated to provide individual assessment of infertility risk factors and ovarian reserve to help women fulfil their reproductive life-plan (Hvidman et al., 2015).

Study design, size, duration: Longitudinal cohort study including the first 570 women aged 20–43 who consulted the FACC at Rigshospitalet, Copenhagen University Hospital from 2011 to 2013. The study aimed to validate the risk assessment score from the initial consultation to identify the main female predictors of prolonged time to pregnancy after two years of follow-up.

Participants/materials, setting, methods: The women answered a baseline questionnaire (demography, health behaviour, gynaecological/reproductive history). A fertility specialist completed a risk assessment score sheet with items on infertility risk factors, AMH and ultrasound measurement of AFC, ovarian volume and pathology. For each parameter the risk score was categorized as green/yellow (low), orange (medium) and red (high) (Hvidman et al., 2015). After two years an email-based questionnaire was distributed regarding subsequent pregnancies, time to pregnancy (TTP), births, failed pregnancy attempts, other health-related or relationship changes.

Main results and the role of chance: A total of 570 women attended the FACC of which 519 (91.1%) answered the follow-up questionnaire. The mean female age was 35 years (SD 4.6), 38% were singles and 75% had an educational length of more than three years. The majority (67.8%, 352/519) tried to conceive within two years, of which 73.6% (259/352) achieved a pregnancy, 21% (74/352) were still trying and 5.4% (19/259) had given up trying. Almost one third (83/259) of the pregnancies were achieved after fertility treatment with IUI-H as the most frequently used procedure among couples (20/49) and IUI-D among singles (19/34). Of the remaining 167 women, 30% wished for a pregnancy at the time of follow-up, but were not actively trying. The women who had tried to conceive ($n = 352$) were similar to the women without any pregnancy attempts regarding age ($P = 0.49$), AMH ($P = 0.28$), AFC ($P = 0.08$), previous pregnancies ($P = 0.87$) and health behaviour. Two thirds of the women with only low risk scores ($n = 62$) conceived spontaneously within 12 months (65.6%), while this figure was only 28% for women with at least one high risk score ($n = 82$). Accordingly, presence of at least one high risk score reduced the odds of achieving a pregnancy within 12 months by 75% (OR 0.25, 95% CI 0.12–0.52).

Limitations, reasons for caution: The women attending the FACC are concerned about their ovarian reserve and reproductive time-span, which could imply a potential selection bias. The homogeneity of the included women and the relatively short follow-up period of two years may impede the predictive value of the continuous variables in the risk assessment.

Wider implications of the findings: The FACC was initiated to provide women information about their current fertility status to prevent involuntary childlessness, infertility and smaller families than desired. Based on these findings the new FACC concept seems usable and offers a tool for fertility experts to guide women on how to fulfil their reproductive life-plan.

Trial registration number: Establishment of the Bio Bank is approved by the Scientific Ethical Committee (nr: H-1-2011-081). Permission to store data was granted by the Data Protection Agency (30-0728).

P-420 Effects on the level of hormone in micro-environment of follicular fluid from advanced oxidation protein products

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Study question: To detect the AOPP level in follicular fluid of women with infetility, and its correlation with estradiol (E_2) level, progesterone (P4) level in follicular fluid.

Summary answer: The follicular fluid AOPP level with different pathogeny was different, higher AOPP level caused lower P concentration in follicular fluid and worse outcome of IVF.

What is known already: Advanced oxidation protein products (AOPP) were the products and mediator of oxidative stress reaction. Its role as a novel marker to investigate oxidation stress has performed in many fields. The AOPP level in body fluid of women with polycystic ovarian syndrome (PCOS) or endometriosis (EMs) were found both higher than health women. In our previous research, AOPP level in follicular fluid presented negative correlations with the proportion of mature oocytes, cleavage rate, fertilization rate, good embryo rate.

Study design, size, duration: 139 women with infertility, including 20 EMs (average age: 33.3 ± 3.9 y; average BMI: 22.27 ± 3.23 kg/m^2), 76 pelvic and tubal factors (average age: 31.8 ± 4.6 y; average BMI: 21.11 ± 2.87 kg/m^2) and 17 PCOS (average age: 28.7 ± 3.0 y; average BMI: 21.98 ± 2.59 kg/m^2) were enrolled. 26 healthy women (average age: 30.6 ± 3.7 y; BMI: 21.02 ± 3.30 kg/m^2) coming to our reproductive technology center because of male factors were recruited as control group.

Participants/materials, setting, methods: Follicular fluid samples were obtained from all groups to measure the levels of AOPP, E2 and P4, and all data about clinical IVF prognosis was collected for analysis. The determination of AOPP was based on the spectrophotometric method. The concentration of A2, E2 and P4 in follicular fluid were determined with the use of commercial Iodine [125I] Radioimmunoassay Kit according to the manufacturer's instructions. Pearson correlation and One-Way ANOVA were applied to analyse data.

Main results and the role of chance: The difference of average AOPP level among groups was significant (PCOS group vs. EMs group: 65.25 ± 25.69 μmol/L vs. 45.73 ± 22.55 μmol/L, $p = 0.021$; PCOS group vs. pelvic and tubal factors group: 65.25 ± 25.69 μmol l/L vs. 51.97 ± 26.08 μmol/L, $p = 0.012$). The correlation between AOPP level and P4 level in follicular fluid was significantly inverse ($r = –0.176$, $p = 0.038$). As well, in pelvic and tubal factors group, AOPP level was negatively correlated with blastocyst rate ($r = –0.294$, $p = 0.032$). In addition, the number of dominant follicles (diameter>14 mm) was positively correlated with the serum P4 level at the time of hCG administration ($r = –0.268$, $p = 0.001$), and significantly negatively correlated with age ($r = –0.377$, $p = 0.000$).
Limitations, reasons for caution: In the future research, the sample size should be amplified further. And the number of samples in different groups is not the same, maybe the results could be influenced by one pathogeny.
Wider implications of the findings: The AOPP in follicular fluid may disturb the endocrine function of granular cells, and lower P4 concentration in follicular fluid. Then high AOPP level in follicular fluid would bring adverse effects on the development and maturity of oocytes.
Trial registration number: This is a basic research.

P-421 ART outcomes for fresh versus differed frozen-thawed day-2 embryo transfer: a matched cohort study

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Study question: To compare assisted-reproductive-technology (ART) outcomes between fresh day-2 embryo transfer (ET) versus differed frozen-thawed day-2 embryo transfer (dif-ET).
Summary answer: ART outcomes were similar for day-2 fresh versus day-2 differed frozen-thawed embryo-transfers.
What is known already: Controlled ovarian stimulation (COS) could potentially have an adverse impact on endometrial receptivity, thereby reducing ART success rates by adversely affecting the implantation step. To address this concern, one emerging strategy is the "freeze-all" approach. Compelling results have been presented that indicate that differed frozen-thawed embryo transfer (dif-ET) is the most suitable method for several sub-populations of ART patients. There nonetheless remains a degree of controversy in this regard. Furthermore, since most of studies have focused on blastocyst transfers, knowledge regarding differed frozen-thawed day-2 embryo transfers has remained quite limited to date.
Study design, size, duration: This cohort study conducted in a tertiary care university hospital, analysed all first transfer/cycles between 01/10/2012 and 31/12/2014. For this study, main inclusion criterias were a day-2 ET and having obtained fewer than 7 zygotes.

Of the initial cohort of 3,116 ART procedures scheduled during the study period, 325 day-2 dif-ET cycles and 1,296 day-2 fresh ET cycles were eligible for matching. The main outcome measure was ongoing-pregnancy rates.
Participants/materials, setting, methods: Fresh and frozen-thawed ET were matched by age and the number of previous ART cycles. Two groups were compared: a group made up of "exposed" women (dif-ET group) who received differed ET using frozen-thawed day-2 ET and an "unexposed" control group (fresh ET group), comprised of women who received fresh day-2 ET. Statistical analyses were conducted using univariate and multivariate logistic regression models.
Main results and the role of chance: A total of 650 cycles were included in the analysis: 325 in the fresh ET group and 325 in the dif-ET group. No significant differences were found between fresh and frozen-thawed ET cycles in terms of clinical pregnancy rates [98/325 (30.15%) vs. 85/325 (26.15%)] and ongoing pregnancy rates [71/325 (21.85%) vs. 60/325 (18.46%)]. In addition, the number of 2PN embryos (3.41 ± 1.58 vs. 3.56 ± 1.72) and the implantation rates (0.20 ± 0.33 and 0.17 ± 0.31) were not significantly different between both groups. Independent predictors for ongoing pregnancy after a multiple logistic regression analysis were the women's age (OR=0.92; 95% CI: 0.87–0.96), BMI (OR=0.94; 95% CI: 0.89–0.99), and the number of 2PN embryos (OR=1.23; 95% CI: 1.08–1.40).
Limitations, reasons for caution: In our center, in fresh ET protocol, day-2 ET was the standard of care; In differed-ET protocol, day-2 ET was done only when less than seven 2PN were obtained. To avoid selection bias, we studied for both groups only day-2 ET derived from ART cycles with less than seven 2PN.
Wider implications of the findings: Embryo-endometrium interaction after a day-2 ET did not appear to be impaired by COS. Given that we performed a broad-based analysis of deferred-ET in this study, further investigation may be required to determine for which specific subgroups of infertile patients deferred-ET may be more efficient and appropriate for ensuring increased-PRs.
Trial registration number: –

P-422 Start of ovarian stimulation on day three versus luteal phase of the menstrual cycle in the same oocyte donor. A prospective observational study

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Study question: Assess ovarian response (oocytes) after ovarian stimulation starting on day 3 or luteal phase of the menstrual cycle in the same oocyte donor.
Summary answer: In the same oocyte donor, the ovarian response is similar when the stimulation is started on day 3 or on the luteal phase of cycle.
What is known already: To optimize the IVF process and obtain sufficient mature oocytes, controlled ovarian hyperstimulation (COH) is needed. Usually, COH is performed from the beginning of the follicular phase on day 2 or 3 of the menstrual cycle. Current findings have described the presence of multiple waves of follicular recruitment within a single ovulatory period. There are publications describing that is possible to obtain mature oocytes starting the COH on luteal phase of cycle. This is an important tool to consider for oncologic patients who want to preserve their fertility and for the programs oocyte donors.
Study design, size, duration: Prospective observational study on a group of 8 oocyte donors from our oocyte donation program between January 2015 and December 2015. Donors who meet the legal requirements and agreed to participate in the study were recruited. Donors were subjected to two cycles of COH, one cycle of conventional COH starting on day 3 (Classical COH) and another on luteal phase after confirming ovulation with ultrasound (Luteal COH), in a 3–5 month interval.
Participants/materials, setting, methods: In the two cycles of stimulation, COH was performed with recombinant FSH at doses of 150–225 IU/day. Administration of GnRH antagonist was started whit a follicle measuring >14 mm. A GnRH agonist was used to trigger ovulation and oocyte retrieval was performed 36 h later. We evaluated count of follicles >10 mm on day 5 of stimulation, follicles >15 mm and estradiol levels on day of agonist GnRH, oocytes and mature oocytes retrieved.
Main results and the role of chance: A total of eight stimulation cycles on Classical COH and eight stimulation cycles on Luteal COH were performed on eight oocyte donors (age 23.38 ± 2.45 years) a 3–5 month interval. No statistically significant differences were found in the COH donors' responses between the Classical COH and Luteal COH starts. The count of follicles >10 mm on day 5 of stimulation was 9.50 ± 3.58 vs. 10.13 ± 2.16, the follicles >15 mm on day agonist GnRH 17.13 ± 7.24 vs. 16.63 ± 5.58, estradiol level (pg/mL) on day agonist GnRH 2,312 ± 1,081 vs. 2,463 ± 954, total days of stimulation 10.25 ± 1.98 vs. 10.25 ± 0.88, total doses of gonadotropin 1959.37 ± 442.38 vs. 1950.00 ± 198.20, oocytes retrieved 23.00 ± 13.09 vs. 19.38 ± 6.11, mature oocytes 21.50 ± 12.37 vs. 17.00 ± 5.37
Limitations, reasons for caution: The limitation of our study could be the small sample size. The study was performed on healthy young women (<35 years), so the results could not be extrapolated to other clinical conditions such as low ovarian reserve, older women, etc.
Wider implications of the findings: The ovarian response in donors is similar when the COH start on early follicular phase or on luteal phase. These results demonstrate that the ovarian stimulation starting in the luteal phase could be useful in patients that want to preserve oocytes o preserve fertility for cancer o social reasons.
Trial registration number: Not applicable

P-423 Incidence of successful pregnancy after weight loss interventions in infertile women: a systematic review and meta-analysis of the literature

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Study question: The impact of weight loss surgery on pregnancy rate.
Summary answer: Our results showed an impressive high incidence (58%) of infertile women who become spontaneously pregnant after surgery.
What is known already: The relationship between obesity and infertility is well established. Increasingly, bariatric surgery is used to treat morbid obesity in women of reproductive age, and data suggest that surgery may have a beneficial influence on fertility. Some studies reported about infertile women who become pregnant after bariatric surgery. However, little is known about the impact of weight loss surgery on pregnancy rate.
Study design, size, duration: A systematic review with meta-analysis of literature has been performed to evaluate the incidence of successful pregnancy after bariatric interventions in infertile women. The primary analysis was the assessment of the incidence of successful pregnancy, which was defined as live birth. Moreover, the impact of major clinical and demographic characteristics on the effect size has been evaluated.
Participants/materials, setting, methods: All studies reporting on the pregnancy rate of infertile women undergoing bariatric surgery were included.

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To be included in the analysis, a study had to provide the incidence of successful pregnancy in infertile women undergoing bariatric interventions. For each study, data regarding sample size, major clinical and demographic variables, and type of bariatric surgery has been extracted.
Main results and the role of chance: By pooling together data from 589 infertile obese women, we have been able to provide an aggregate estimation of successful pregnancy after weight loss interventions. Our results showed an impressive high incidence (58%) of infertile women who become spontaneously pregnant after surgery. A meta-regression approach showed an inverse association between baseline Body Mass Index (BMI) values and the weighted mean incidence of successful pregnancy after bariatric surgery (Z-score − 3.27, p = 0.001). In addition, an inverse association was found between BMI reduction after surgery and the Weight Mean Incidence (WMI) of successful pregnancy (Z-score − 2.96, p = 0.003).
Limitations, reasons for caution: Conclusion drawn from the current literature must be interpreted with caution owning to the quality of the original data provided by included studies: over 60% of the conclusions originated from a single series, time to pregnancy was not reported, and only mean post-operative weight loss was informed.
Wider implications of the findings: Based on our results, bariatric surgery has been found to be associated with a high incidence of successful pregnancy in infertile women, providing the rationale to guide clinical practice for this challenging problem and direct future basic science and translational research.
Trial registration number: Not required

P-424 Effect of female body mass index on oocyte quantity: treatment cycle number is a possible effect modifier

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Study question: Does treatment cycle number influence the outcome when investigating the effect of female body mass index on oocyte quantity in IVF?
Summary answer: The association between female BMI and oocyte outcome may be modified by cycle number, BMI having an adverse effect in first but not subsequent cycles.
What is known already: The literature regarding obesity and the impact on oocyte outcome is conflicting and it varies studies between whether they report on several treatment cycles per women or only on first treatment cycle. Overweight and obese women may require higher doses of gonadotropin when undergoing IVF. Consequently one may expect a sub-optimal oocyte retrieval in the first treatment cycle and thus a larger compensation in gonadotropin-dose in the following treatment-cycles and a more favorable outcome.
Study design, size, duration: Historical cohort study on 5,342 ART- cycles performed at the Fertility Clinic, Aarhus University Hospital during the period 1999–2009.
Participants/materials, setting, methods: Women were included in the study if they were receiving either *in vitro* Fertilization (IVF) or Intra Cytoplasmatic Sperm Injection (ICSI) treatment. Exclusion criteria were missing information on BMI or treatment type. Further, women were excluded if they had ovulated before oocyte retrieval. According to baseline BMI, women were divided into four categories following the WHO standards. Multiple linear regressions analyses were performed accounting for the non-independence of ≥2 cycles in a woman.
Main results and the role of chance: No statistically significant differences were observed in oocyte yield for underweight, overweight and obesity compared to normal weight women when analyzing all treatment cycles. Overweight women had significantly fewer mature (MII) oocytes (p = 0.009) than normal weight women, whereas no differences was observed for underweight and obese women. Stratifying according to cycle number revealed a more sub-optimal outcome in first treatment cycles than in the following cycles, suggesting a possible interaction or effect modification from cycle number or a factor related to cycle number. The median dose of total FSH given to the four BMI groups could not straight forwardly explain the less optimal oocyte outcome observed in first treatment cycles.
Limitations, reasons for caution: A relative large number of cycles were excluded due to lack of information regarding either BMI or treatment type. Though, one might assume that the lack of information in the excluded cycles was due to chance, it is not possible to assess whether this could have influenced the results.
Wider implications of the findings: The findings of our study indicate that cycle number may be a possible effect modifier for the association of BMI and oocytes. This could have implications for future studies on BMI and the chance of achieving pregnancy.
Trial registration number: Not applicable

P-425 Saline infusion sonohysterography is required for the initial workup of infertility

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Study question: The aim of this retrospective study was to accurately assess the diagnostic accuracy of Transvaginal sonography (TVS), and Saline infusion sonohysterography (SIS).
Summary answer: As long as hysteroscopy is not feasible for every patient the addition of SIS to TVS reduces both false positive and false negative of TVS.
What is known already: The assessment of the uterine cavity is one of the early steps in infertility workup. The gold standard for diagnosis is hysteroscopy, however currently it is still not feasible to offer this procedure to all patients. In order to reduce hysteroscopy burden different centers have used the following strategies: 1) some centers offer Transvaginal sonography (TVS), and if abnormal followed by Saline infusion sonohysterography (SIS). Based on the results of SIS a decision regarding hysteroscopy is made. 2) Others perform hysteroscopy directly after abnormal TVS. Current data provide conflicting results regarding the sensitivity and specificity of TVS an SIS.
Study design, size, duration: In the current study we assessed retrospectively whether the addition of SIS provides any diagnostic advantages to TVS. We assessed 564 women who underwent TVS, SIS and hysteroscopy between January 2009–July 2015. Mean age was 37.5 years (range 23–51)
Participants/materials, setting, methods: Using the Quality Assessment of Diagnostic Accuracy Studies (QUADAS tool) we aimed to compare accuracy between procedures done in the follicular vs. ovulatory/luteal menstrual phases, as well as to evaluate the diagnostic accuracy variance with time interval, using hysteroscopy as the gold standard, in the diagnosis of uterine cavity abnormalities and malformations in a population of sub-fertile female patients.

Main results and the role of chance: Our results provide evidence that SIS is more accurate than TVS (accuracy SIS=0.8 TVS=0.56 $p < 0.01$). Specifically the sensitivity of SIS to diagnose polyps and intrauterine adhesion (IUA) was statistically significant higher. The accuracy of SIS for diagnosing IUA and polyps decreased as the time from SIS to hysteroscopy increased.
Limitations, reasons for caution: The study was retrospective, and since there were no clear guidelines in our clinic to perform all tests to all patients not all patients in our clinic underwent all three studies. Accordingly a selection bias for patients undergoing all workup might have occurred.
Wider implications of the findings: Hysteroscopy is still expensive and requires experienced physicians. Our results provide evidence to the usefulness of SIS in the setting of lack of resources for hysteroscopy. These results can change the standard of care in many places over the world.
Trial registration number: N/A

P-426 Are extremely high progesterone levels still an issue in IVF?

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Study question: with advances in freezing/thawing techniques, the "freeze-all" strategy might overcome detrimental effects of extremely high progesterone (P) levels during Controlled Ovarian Stimulation (COS).
Summary answer: results support the "freeze-all" strategy in cases of extremely high P levels during COS. Cycle cancellation due to premature luteinization should therefore be avoided.
What is known already: the "freeze-all" strategy – where the entire cohort of embryos/blastocysts is cryopreserved for subsequent Frozen-thawed Embryo-Transfer (FET) – is already considered the preferred method for management of subtle P elevation during COS. However, published studies have never focused on extremely high P levels. While slight P elevation relates to ovarian response to COS, extremely high P represents the occurrence of premature luteinization. The scant available literature suggests that cycle cancellation is still the preferred method of dealing with extremely high P levels as caused by accidental ovulation. With advances in freezing/thawing techniques, however, cycle cancellation might be avoided.
Study design, size, duration: the study is a non interventional, retrospective, case-control, single-center trial conducted on COS cycles complicated by extremely high P levels. All patients underwent a "freeze-all" program at the blastocyst stage between January 2012 and September 2014. Controls ($n = 67$) were matched to cases ($n = 42$) for age, BMI, basal FSH and duration of infertility. Data on clinical/ongoing pregnancy rates and cumulative clinical/ongoing pregnancy rates were obtained from all FETs performed up to November 2015.
Participants/materials, setting, methods: patients undergoing a "freeze-all" program due to premature luteinization during COS – identified by extremely high P levels at induction (P levels ≥3.0 ng/ml and/or P/Estradiol ratio ≥1) – were compared to controls undergoing a "freeze-all" program with P at induction <1.5 ng/ml. After blastocyst culture, FET was performed (maximum 2 blastocysts transferred per attempt). Power analysis was used to have 80% power to detect a 50% difference in blastulation rates.
Main results and the role of chance: Number of oocytes retrieved was similar between Cases and Controls (12.6 ± 6.5 vs. 15.0 ± 6.8 respectively, Mean ± SD, $p = 0.06$) and so was percentage of mature oocytes (Metaphase two or MII oocytes, 71.6% ± 21.6% vs. 69.7% ± 20.6% respectively, Mean ± SD, $p = 0.20$). Also blastulation rate was not decreased in patients with premature luteinization compared to controls (48.1% ± 20.5% in Cases vs. 52.3% ± 24.9% in Controls, $p = 0.36$) and the proportion of top-quality blastocysts was as well comparable between cases and controls (14.3 ± 25.2 vs. 12.7 ± 21.4 respectively, Mean ± SD, $p = 0.72$). Ongoing pregnancy rates after the first FET (38.1% in Cases and 41.0% in Controls, $p = 0.83$) and cumulative ongoing pregnancy rates after three FET cycles (40.5% in Cases vs. 47.8% in Controls, $p = 0.55$) were as well similar. Based on this, COS cycle cancellation – which is likely the most frequently used method for management of extremely high P levels during COS- currently represents a clinical misconduct.
Limitations, reasons for caution: the relatively small number of patients included represents a limitation of our study and further validation in larger multicenter trials would be warranted. Also, a "freeze-all" strategy as suggested by our study should only be undertaken by centers with the appropriate freezing/thawing facilities and expertise.
Wider implications of the findings: premature luteinization doesn't have consequences at oocyte/embryo level in a "freeze-all" strategy. Cycle cancellation in case of very high P levels before induction, at present, represents a malpractice. This also supports the "random start" in COS for fertility cryopreservation and suggests that P assessment might be useless in segmented cycles.
Trial registration number: n/a

P-427 The impact of premature progesterone rise on the outcome of intrauterine insemination cycles with controlled ovarian hyperstimulation in unexplained infertility

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Study question: What is the impact of premature progesterone rise on the day of hCG administration on cycle outcomes in terms of live birth in COH-IUI cycles?
Summary answer: Premature progesterone is a frequent feature of COH-IUI cycles and associated with decreased live birth rates.
What is known already: Addition of COH to IUI increases the number of oocytes available for fertilization and the risk of premature progesterone rise. Most of the data with regard to the impact of premature P rise were acquired from COH cycles in IVF and it was shown that different cutoff levels of premature P rise ranging between 1–1.5 ng/mL on the day of hCG has detrimental effect of IVF outcome. However, there were a few studies which investigate the incidence and the impact of early P rise in COH-IUI cycles.
Study design, size, duration: Six hundred forty two cycles of 460 couples with unexplained infertility having COH-IUI treatment between 2008–2013 in an university-based tertiary infertility clinic were enrolled in this prospective study.
Participants/materials, setting, methods: Couples with unexplained infertility having COH-IUI treatment with a starting dose of 75 IU recombinant FSH were enrolled. Serum P levels were determined on the day of hCG trigger. Premature P rise was defined as progesterone ≥1 ng/ml. The primary outcome measure was live birth per cycle with regard to P levels of ≥1 ng/mL and ≥1.5 ng/mL. Secondary outcome measures were cycle characteristics associated with P rise.
Main results and the role of chance: The incidence of premature P rise was 18.0%. P levels on hCG day were significantly lower in cycles with live birth as compared to cycles without live birth 0.48 ± 0.50 vs. 0.64 ± 0.72. Live birth rates were significantly lower in cycles with hCG day P levels ≥1.0 ng/dL (7% vs. 16.2%) and ≥1.5 (5.7% vs. 15.4%). It was found that P levels on the day of hCG trigger was the only significant variable to predict live birth on multivariate analysis in comparison to age, number of dominant follicles, estradiol, LH levels on the day of hCG trigger. The number of dominant follicles on hCG day and premature LH surge were the only significant variables related with premature P rise.
Limitations, reasons for caution: There has also been no exact strategy to prevent P rise in COH cycle; so screening of hormone levels should not been recommended as a standard practice in COH- IUI cycles.
Wider implications of the findings: The negative effect of progesterone levels of both 1 ng/mL and 1.5 ng/mL on the day of hCG was observed in COH-IUI cycles. COH-IUI outcomes might be improved by avoiding LH surge and excess follicular development.
Trial registration number: Absent

P-428 Maternal serum bile acid composition at conception is related to birth weight of offspring

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Study question: Does the bile acid (BA) composition of maternal serum and follicular fluid (FF) of women undergoing IVF relate to birth weight of the offspring?
Summary answer: There is an association between serum levels, but not FF levels, of primary BA and ursodeoxycholic acid (UDCA) at conception and offspring birth weight.
What is known already: We have previously reported the presence of BA in FF of human ovaries and the relationship between FF BA and embryo development, suggesting a role of BA in fertility. Furthermore, it has been described that human offspring who encountered a cholestatic condition during their fetal development may have an increased incidence of preterm birth and low birth weight. These offspring may also show metabolic syndrome related phenotypes during adolescence. The influence of the maternal serum and FF BA pool at the moment of conception on characteristics of offspring at birth has yet to be explored.
Study design, size, duration: In our retrospective cohort study, FF and serum samples collected in the setting of a study on the effectiveness of modified natural cycle-IVF (MNC-IVF) (January 2001 to June 2004) were used. Only samples from cycles in which one oocyte was obtained were included, assuring that the FF BA level was related to the embryo that was transferred. Furthermore, only samples from IVF patients who achieved a singleton live birth were used.
Participants/materials, setting, methods: Total BA and BA subspecies were measured in serum and FF samples by enzymatic fluorimetric assay and liquid chromatography-mass spectrometry, respectively. The association of total BA and BA species with birth weight was analysed and, in a regression analysis, corrected for offspring gender, gestational age at birth, BMI of mother, parity and maternal smoking during pregnancy.
Main results and the role of chance: Serum was available from 51 patients fulfilling the inclusion criteria and FF from 34. Serum levels of the primary BA chenodeoxycholic acid (CDCA) and cholic acid (CA) and of the BA UDCA were significantly correlated with birth weight ($r = -0.395$, $P = 0.004$; $r = -0.311$, $P = 0.026$; $r = -0.319$, $P = 0.023$, respectively). Neither the secondary BA deoxycholic acid and lithocholic acid, nor total BA levels were correlated with birth weight. After correction for possible confounders, CDCA, CA and UDCA were still significant predictors of birth weight ($P = 0.002$, $P = 0.004$ and $P = 0.048$, respectively), while total and secondary BA remained unrelated. For FF total BA and BA species no significant correlation with birth weight was found. Taken together, these novel data suggest that even in non-cholestatic individuals maternal serum BA levels are related to fetal growth. As the serum BA levels remain stable during pregnancy and within the normal pre-conceptional range, the effect on fetal growth is probably induced during pregnancy.
Limitations, reasons for caution: Despite the limited number of investigated samples and participants, we believe that the robustness of the correlation even after correction makes these data worth reporting.
Wider implications of the findings: Metabolic (programming) effects of BA are an emerging topic supported by a variety of data. Further steps should be taken to unravel the biological mechanism of BA in oocyte and foetal development.
Trial registration number: N/A

P-429 Effect of cancer and its treatment on reproductive function markers in long-term female survivors of childhood cancer: results of the Dutch nationwide DCOG LATER-VEVO study

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Study question: Which chemotherapeutic agents and radiation sites are dose-dependently associated with reproductive function markers in female childhood cancer survivors (CCSs)?
Summary answer: Cyclophosphamide, procarbazine, etoposide and vincristine as well as ovarian radiation dose were associated with low AMH levels and antral follicle counts in a dose-dependent manner.
What is known already: Late effects of childhood cancer treatment have emerged as a growing concern, since survival has dramatically increased. Adverse late effects on the reproductive system may not become apparent until many years later. Anti-cancer treatment, often consisting of a combination of radiotherapy and chemotherapy, may reduce the fertile life span and induce premature menopause, as therapy may deplete the non-renewable pool of primordial follicles in the ovaries. Alkylating agents and procarbazine have been associated convincingly with gonadotoxicity. The effect of dose, as well as the effects of age at time of treatment and the time since treatment, is less well known.
Study design, size, duration: The study is part of the DCOG LATER-VEVO study, a nationwide retrospective cohort study on female fertility of Dutch CCSs. The control group consisted of sisters of survivors and females from the general population. Data collection took place between January 2008 and May 2014.
Participants/materials, setting, methods: The study population consisted of female 5-year CCSs who were treated between 1963 and 2002 in the Netherlands, and who were at least 18 years at inclusion. Of the 1,166 CCSs and 836 controls who participated in the study, 629 (54%) and 432 (52%), respectively, provided a blood sample and/or underwent ultrasonic evaluation of the ovaries. Both multivariate linear and logistic regression analyses were performed.
Main results and the role of chance: CCSs appeared to be at increased risk of having low (<0.5 µg/l) AMH levels and low number of antral follicles (≤5 follicles) (OR (95% CI) 12.4 (3.7–41.5) and 3.7 (1.2–11.3), respectively) compared to healthy controls. Results show that having been treated before menarche in general seemed less damaging compared to being treated during and after menarche, with AMH and AFC levels being higher when treated before menarche. In a multivariate model, corrected for age, BMI, use of contraceptive therapy and time since treatment, there was a statistically significant inverse dose-effect relation of cyclophosphamide ($p = 0.001$), procarbazine ($p < 0.001$), vincristine ($p = 0.003$) and etoposide ($p = 0.01$) on AMH levels. In addition, increasing radiation doses on the abdomen or pelvis ($p < 0.001$) or total body ($p < 0.001$) was associated with low AMH levels. Furthermore, increasing doses of cyclophosphamide ($p = 0.001$), procarbazine, ($p < 0.001$) and higher dose of radiotherapy to the abdomen or pelvis ($p < 0.001$) and total body irradiation ($p < 0.001$) were significantly associated with lower AFCs.
Limitations, reasons for caution: AMH and AFCs are considered reliable markers for ovarian reserve, however their predictive value for pregnancy remains questionable. Premature menopause would be better outcome to assess this research question. However, as the median attained age of the study

population was 28–30 years, the cumulative incidence of premature menopause was low.
Wider implications of the findings: The results of the study improve the physician's ability to counsel female CCSs on family planning. In addition, females who are about to undergo gonadotoxic cancer therapy may benefit since proven high risk populations can be offered clinical interventions aimed at preserving a women's reproductive function.
Trial registration number: NTR2922 http://www.trialregister.nl/trialreg/admin/rctview.asp?TC=2922

P-430 Outcomes of blastocyst transfer program resulting from vitrified donor oocytes

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Study question: What is the clinical pregnancy rate and live birth rates per warmed oocyte used in an egg recipient program compared to the use of fresh autologous oocytes? Is there a difference in the obstetrical outcomes between the two groups?
Summary answer: Blastocysts from vitrified oocytes have good implantation potential resulting in a live birth rate comparable to fresh oocytes with no difference in neonatal outcomes.
What is known already: Oocyte donation is a successful and well-established treatment of age-related female infertility, where the oocyte and subsequent embryo qualities are optimized by donated oocytes from young women resulting in high pregnancy rates. Since the introduction of vitrification, the use of vitrified donor oocytes enables the clinician better coordination for treatment of the egg recipients. The clinical pregnancy rates using vitrified donor oocytes is comparable to the use of fresh oocytes.
Study design, size, duration: A prospective case control study comprising of 220 patients from January 2010 to April 2015. 1127 oocytes were vitrified from 110 egg donors at the Centre of Reproductive and Genetic Health (CRGH) clinic. The control group were age matched controls who underwent ICSI treatment with their own oocytes ($n = 1165$).
Participants/materials, setting, methods: 110 egg donors went through a stimulation cycle at CRGH resulting in 1127 vitrified oocytes. The control group consisted of age matched controls, who underwent ICSI with fresh oocytes. The clinical and obstetrical outcomes were compared in both the groups. Summary data were obtained and logistic regression analysis performed using SPSS Version 23 and Stata 2015. Case and control percentages were compared by Fisher's exact test. A significance level of 5% was adopted throughout.
Main results and the role of chance: The survival rate of vitrified oocytes was 74.1% (95% CI: 71.5–76.6). The clinical pregnancy rate (per embryo transfer) using vitrified oocytes was found to be 48.4% compared to 47.3% in the control group. The live birth rate per warmed oocyte was found to be 6.8%. The live birth rate per embryo transfer in the vitrified oocyte group was 41.5% (95% CI 29.3–54.9) compared to 42.9% (95% CI 31.9–54.5) in the control group. There was no difference in the gestational age of the infants in both the groups [39 weeks (95% CI 33–42) vs. 40 weeks (29–42), $p = 0.27$]. There was no difference in the gestational weight at birth in both the groups (3175 grams (1616–4480) vs. 3289 (1502–4337) $p = 0.35$).
Limitations, reasons for caution: There was no relation between the number of allocated donor eggs with livebirth rate. Larger studies are required to establish the safety and efficacy of oocyte cryopreservation in women requesting social freezing.
Wider implications of the findings: This study reiterates the safety and efficacy of using vitrified donor oocytes in a recipient program and demonstrates no difference of obstetrical outcomes in both groups. The findings of this study could be extrapolated to women opting for egg freezing due to reasons like underlying malignancy or social reasons.
Trial registration number: Not applicable

P-431 Alteration in ovarian expression of bone morphogenetic protein 15, growth differentiation factor 9, and c-kit according to female aging in mice

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Study question: Can ovarian expression of bone morphogenetic protein 15, growth differentiation factor 9, and c-kit be a new standard marker for the objective evaluation of ovarian aging resulted from female aging?
Summary answer: The ovarian expression of bone morphogenetic protein 15, growth differentiation factor 9, and c-kit can be a standard marker of ovarian aging.
What is known already: Although many studies have showed that antral follicle count (AFC) and anti-Müllerian hormone (AMH) has been suggested as good predictors of ovarian reserve, more researches are needed to standardize these threshold values universally. It has been well known that bone morphogenetic protein 15 (BMP15), growth differentiation factor 9 (GDF9) are oocyte-secreted growth factor required for ovarian follicle development and ovulation in mammals. c-Kit has been also known to play an important role in follicle growth as a close coordinator secreted by granulosa cells. However, the alteration in ovarian expression of these factors according to the ovarian aging is not determined yet.
Study design, size, duration: The ovarian expression of BMP15, GDG9 and c-kit were evaluated according to the female aging (10, 20, 30, and 40 weeks-old) by RT-PCR, real-time PCR, western blot and immunohistochemistry. In addition, follicle count, such as primordial, primary and secondary follicle, and the oocyte developmental competency were also examined as a traditional marker for ovarian aging.
Participants/materials, setting, methods: Ovaries of differently aged C57BL female mice (from 10 to 40 weeks-old) were provided in the evaluation of follicle count and expression of BMP15, GDF9, and c-kit. Also these aged mice were superovulated with PMSG and hCG, and then immediately mated with an individual male. Zygotes were obtained and cultured for 5 days. The number of oocytes ovulated and the developmental rate to blastocyst were investigated.
Main results and the role of chance: All real-time PCR, western blot and immunohistochemistry showed a decreased ovarian expression of BMP15, GDF9, and c-kit in a aging-dependent manner. Accompanied by this result, the follicle count, the number of ovulated oocytes, and developmental rate were significantly decreased in aged mice (30 and 40 weeks old) compared to young mice (10 weeks old).
Limitations, reasons for caution: This study do not show that these results could equally applicable to the human study and these facotrs can be a good marker for human ovarian aging.
Wider implications of the findings: Recent data indicate that ovarian expression of BMP15, GDF9, c-kit can be a standard marker for the evaluation of ovarian aging. This result may be very useful standard marker when examining whether a candidate material shows an anti-aging effect on female reproductive aging.
Trial registration number: No

P-432

Abstract withdrawn by the author

P-433 The long non-coding RNA nuclear-enriched abundant transcript 1 is downregulated in granulosa cells from women with advanced age

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Study question: To analyze the expression level of the long non-coding RNA (lncRNA) nuclear-enriched abundant transcript 1 (NEAT1) in human granulosa cells and analyze its role in the ageing ovary.
Summary answer: The expression of NEAT1 is significantly decreased in women with advanced maternal age, which is localized in the nuclei of human granulosa cells.
What is known already: Neat1 is a non-protein-coding RNA that constitutes nuclear bodies known as paraspeckles, nuclear domains implicated in mRNA nuclear retention. Cell-based studies indicate that Neat1 is a crucial regulator of gene expression. Neat1 knockout mice stochastically fail to become

pregnant despite normal ovulation, and corpus luteum dysfunction may be the primary causes of the decreased fertility. Studies on gene expression and regulation in human granulosa cells are of interest due to their potential for estimating the oocyte viability and *in vitro* fertilization (IVF) success. However, studies on lncRNA level in the human ovary have been scarce, especially in ageing granulosa cells.

Study design, size, duration: In this study, we described lncRNA profile in mural granulose cells isolated from sixty patients undergoing routine IVF treatment.

Participants/materials, setting, methods: Sixty patients (31 women <35 years with normal ovarian reserve and 29 women ≥38 years) referred to our center for IVF were included in this study after obtaining written informed consent. Gene expressions were examined by real-time quantitative reverse transcription-PCR in granulose cell collected from follicular fluid. Cell fractionation was performed to isolate nuclear and cytoplasmic RNAs using a Paris kit.

Main results and the role of chance: There was no difference between patients in the two groups in terms of body mass index (BMI), serum concentration of luteinizing hormone (LH), folliclestimulating hormone (FSH), prolactin (PRL), estradiol (E2), progesterone (P) and total testosterone (TT). Compared to women <35 years with normal ovarian reserve, the number of eggs retrieved (10.74 ± 0.54 vs. 7.69 ± 0.70, $P = 0.001$) and the expression level of NEAT1 (1.40 ± 0.14 vs. 0.82 ± 0.08, $P = 0.001$) was significantly lower in women with advanced maternal age. A negative correlation was observed between the NEAT1 expression and the age ($r = -0.336$, $P = 0.009$).

Furthermore, NEAT1 was found to localized in the nuclei of human granulosa cell tumor cells (KGN) as expected.

Limitations, reasons for caution: The data on lncRNA NEAT1 are preliminary and are further investigated. Additional molecular studies regarding the function and mechanism of abnormal expression of NEAT1 in granulosa cells will be performed.

Wider implications of the findings: Our results indicate that reduced expression of the nuclear long noncoding RNA NEAT1 is potentially associated with ageing ovary. To reveal the function of lncRNA NEAT1 in ovary will provide novel insights into reproductive ageing.

Trial registration number: NA.

P-434 Efficacy and safety of follitropin delta in an individualised dosing regimen: a randomised, assessor-blind, controlled phase 3 trial in IVF/ICSI patients (ESTHER-1)

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Study question: To evaluate ovarian response, ongoing pregnancy and implantation rates, and safety outcomes of follitropin delta (FE 999049; recombinant FSH preparation produced from the human cell line PER.C6®) administered according to an individualised dosing regimen based on anti-Müllerian hormone (AMH) and body weight in comparison to follitropin alfa in first cycle IVF/ICSI patients.

Summary answer: Follitropin delta in an individualised fixed-dose regimen yields non-inferiority versus follitropin alfa in ongoing pregnancy/implantation, more patients with appropriate ovarian response and reduced OHSS risk.

What is known already: Identical IU doses (in-vivo rat Steelman-Pohley bioassay) of follitropin delta and follitropin alfa do not result in comparable pharmacokinetic and pharmacodynamic profiles in humans. Therefore, follitropin delta doses are expressed in protein content (µg) and not by bioactivity (IU). The follitropin delta dosing regimen stratifies patients according to response potential and applies an individualised dose based on an ovarian biomarker (AMH) and a patient characteristic (body weight). This approach constitutes an opportunity for safer stimulation without compromising success rates.

Study design, size, duration: Randomised, assessor-blind, controlled trial in 1,326 women, 18–40 years, undergoing their first IVF/ICSI cycle. Patients underwent single blastocyst transfer except those 38–40 years without good-quality blastocysts who had double blastocyst transfer. Ongoing pregnancy and ongoing implantation were assessed 10–11 weeks after transfer (co-primary endpoints; prespecified non-inferiority margin: lower limit (LL) of 95% confidence interval (CI) >−8.0%).

Participants/materials, setting, methods: The daily follitropin delta dose was individualised and fixed throughout stimulation – AMH <15 pmol/L: daily dose of 12 µg, AMH ≥15 pmol/L: daily dose decreasing from 0.19 to 0.10 µg/kg body weight by increasing AMH (maximum daily dose 12 µg). Elecsys® AMH, Roche Diagnostics was used. The daily follitropin alfa dose was 150 IU (11 µg) for the first five days and could thereafter be adjusted by the investigator according to the patient's response.

Main results and the role of chance: The number of oocytes retrieved was similar for follitropin delta and follitropin alfa in the overall population (10.0 and 10.4, respectively). However, more ($p < 0.05$) patients using the individualised fixed-dose regimen of follitropin delta obtained 8–14 oocytes, despite dose adjustments in 36.8% of follitropin alfa patients in contrast to 0% of follitropin delta patients. Among patients with AMH <15 pmol/L, treatment with follitropin delta was associated with more ($p < 0.05$) oocytes compared to follitropin alfa (8.0 vs. 7.0, $p < 0.05$) as well as a lower ($p < 0.05$) incidence of patients with <4 oocytes. Among patients with AMH ≥15 pmol/L, treatment with follitropin delta was associated with fewer ($p < 0.05$) oocytes compared to follitropin alfa (11.6 vs. 13.3, $p < 0.05$) as well as a lower ($p < 0.05$) incidence of patients with ≥15 or ≥20 oocytes. OHSS and/or OHSS preventive interventions occurred less ($p < 0.05$) frequently in patients receiving an individualised dose of follitropin delta than in patients in the follitropin alfa group. The number of blastocysts obtained was comparable between follitropin delta and follitropin alfa (3.3 and 3.5, respectively). Non-inferiority was established for ongoing pregnancy (30.7% vs. 31.6%; LL 95% CI −5.9%) and ongoing implantation (35.2% vs. 35.8%; LL 95% CI −6.1%) for follitropin delta compared to follitropin alfa.

Limitations, reasons for caution: The individualised dosing regimen based on the patient's serum AMH and body weight is developed specifically for follitropin delta due to its differential pharmacokinetic and pharmacodynamic profiles. The regimen documented in this confirmatory trial is derived from prospective investigation in a phase 2 dose-response trial followed by modelling and simulation.

Wider implications of the findings: This trial demonstrates the clinical relevance of tailoring fertility treatment in a personalised/stratified medicine approach. By application of an ovarian biomarker (AMH) and a patient characteristic (body weight), individualised dosing of follitropin delta in a simple fixed-dose regimen provides more appropriate ovarian response and improved OHSS risk management.

Trial registration number: NCT01956110

P-435 Long-term melatonin treatment for the prevention of ovarian aging

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Study question: Does the long-term melatonin treatment prevent ovarian aging in mice?

Summary answer: Long-term melatonin treatment increased the number of ovulated oocytes and fertilization rates. The present results indicate that melatonin prevents ovarian aging in mice.

What is known already: Ovarian aging occurs as a result of a decrease in the number and quality of oocytes in ovarian cortex follicles. Oxidative stress caused by reactive oxygen species (ROS) is thought to be major cause of ovarian aging. However, there are no established treatments to prevent ovarian aging. On the other hand, melatonin, which is a hormone secreted by a pineal gland, is known as a powerful free radical scavenger and a broad-spectrum antioxidant.

Study design, size, duration: Female ICR mice (10 weeks old) were divided into two groups: half were fed with water as a control (control group: C) and the other half were supplemented with 100 µg/ml melatonin water (melatonin group: M) until 43 weeks. Mice were superovulated by PMSG and hCG injection. Oocytes and ovaries were collected 15 h after hCG injection.

Participants/materials, setting, methods: 1) Oocytes were recovered from the oviduct. 2) *in vitro* fertilization was performed using spermatozoa from male ICR mice. 3) The ovaries were fixed in 4% paraformaldehyde, and serially sectioned in 5 mM slices and stained with hematoxylin–eosin. 4) Transcriptome changes in the ovaries were analyzed by microarray. This study was approved by the ethics committee for animal experimentation.
Main results and the role of chance: 1) The number of ovulated oocytes was decreased (33 week: 10.4 ± 6.4, 43 week: 6.4 ± 1.1) during aging process in C. However, the number of ovulated oocytes was greater (33 week: 19.0 ± 10.0, 43 week: 11.6 ± 3.2) in M compared with C. 2) The fertilization rate was higher in M (33 week: 67.4%, 43 week: 50.9%) compared with C (33 week: 52.9%, 43 week: 21.4%). 3) The number of primordial follicle was higher in M compared with C. 4) The transcripts decreased with aging and increased by melatonin treatment were detected, and ribosome-related genes and free radical scavenging network were identified.
Limitations, reasons for caution: The study used the mouse as a model and the applicability of the observed phenomena in humans warrants further investigation.
Wider implications of the findings: Many women are suffering age-related infertility because the age of marriage has increased. Long-term melatonin treatment may become a new management to prevent the decrease in number and quality of oocytes with age.
Trial registration number: non

P-436 Unique geometry of sister kinetochores in human oocytes during meiosis I may explain maternal age-associated increases in chromosomal abnormalities

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Study question: How are sister kinetochores in human oocytes arranged to enable the co-segregation of sister chromatids at the first meiotic division?
Summary answer: Sister kinetochores in human oocytes appear separated during meiosis I and are each able to act as separate attachment sites for microtubule fibres.
What is known already: Human oocytes are highly prone to chromosome segregation errors at the first meiotic division. During this division, sister chromatids segregate together, which requires kinetochores on sister chromatids to form attachments to spindle kinetochore-fibres (k-fibres) emanating from the same pole of the spindle. Of the meiotic sister kinetochores that have been studied so far, in maize, yeast and mouse, all appear to be fused and act as a single functional unit. However, in humans oocytes, the arrangement of sister kinetochores and the mode of k-fibre attachment have not been characterised.
Study design, size, duration: Fixation and immunofluorescence of whole human MI oocytes donated by women undergoing *in vitro* fertilisation or intracytoplasmic sperm injection (IVF/ICSI).
Participants/materials, setting, methods: Oocytes no longer required for treatment were donated by women undergoing IVF/ICSI at the Centre for Reproductive Medicine at University Hospital Coventry & Warwickshire. Meiotic metaphase I (MI) stage human oocytes were fixed in paraformaldehyde and immunofluorescence was performed using primary antibodies targeted to kinetochores (CREST, Bub1 and CENP-E) and microtubules (α-tubulin). Cold-shock treatment was used to visualise k-fibre attachments. High-resolution 3D image stacks of the oocyte chromosomes were acquired by spinning-disk confocal microscopy.
Main results and the role of chance: Analysis of over 1900 kinetochores stained with CREST revealed that the majority (78%) of sister kinetochores pairs in human MI oocytes appear as two distinct spots, indicating that sisters are not physically fused. Markers for the outer (Bub1) and fibrous corona (CENP-E) regions of the kinetochore also appeared separated, indicating that the entire kinetochore is distinct between sisters. The presence of dual k-fibre attachments, in which each kinetochore within a pair had formed an attachment to a spindle k-fibre, indicated that sister kinetochores can act as separate attachment sites. Notably, with increasing female age, the separation between kinetochores increased, from a mean of 0.65 µm in women under 33 to 0.79 µm in those over 38 ($p < 0.0001$, unpaired t-test). This is indicative of a decline in centromeric cohesion with female age.
Limitations, reasons for caution: Human MI oocytes available to research are those that did not mature *in vivo* and come from a population of women experiencing fertility problems.
Wider implications of the findings: The increasing separation of sister kinetochores and their ability to act as independent attachment sites may explain the high proportion of unstable attachments that form in MI and thus indicate why human oocytes are prone to aneuploidy, particularly with increasing maternal age.
Trial registration number: N/A

P-437 Effect of body mass index on the outcomes of controlled ovarian hyperstimulation in Chinese women with polycystic ovary syndrome: a multicenter, prospective, observational study

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Study question: What is BMI distribution of Chinese women with PCOS and whether BMI influence the outcomes of IVF/ICSI techniques in this population?
Summary answer: More than one-third of Chinese women with PCOS are overweight or obese. Elevated BMI associated with higher rFSH dosage requirement and lower pregnancy rate.
What is known already: IVF/ICSI with controlled ovarian hyperstimulation is important option for women with infertility due to PCOS. Studies conducted outside China have reported that 35–65% of patients with PCOS are obese, and that BMI can influence the success rate of IVF/ICSI techniques. However, BMI distribution varies in different ethnic populations. No large scale study has been reported on BMI and its influence on IVF outcomes in Chinese PCOS women.
Study design, size, duration: This was a multicenter, prospective, observational study conducted at 9 hospitals in China between August 2011 and April 2013. Of 801 subjects initially enrolled, total 774 PCOS patients underwent IVF/ICSI were included into the intention to treat (ITT) analysis and observed through one IVF/ICSI cycle.
Participants/materials, setting, methods: The study enrolled women (aged 20–35 years) with PCOS undergoing IVF/ICSI, using rFSH and a long down-regulation protocol. The primary outcome was BMI distribution (underweight, <18.5 kg/m^2; normal, 19–23.9 kg/m^2; overweight, 24–27.9 kg/m^2; obese, ≥28 kg/m^2). Secondary outcome measures, including baseline demographic characteristics, laboratory biochemical tests, rFSH dosage, ovulation and pregnancy outcomes and adverse events were compared among different BMI groups.
Main results and the role of chance: Among 774 subjects (age, 27.9 ± 3.1 years; BMI, 23.0 ± 3.5 kg/m^2), 51 (6.6%) were underweight, 449 (58.0%) normal weight, 211 (27.3%) overweight, and 63 (8.1%) obese. When compared between groups with different BMIs, no statistical difference was detected in basal testosterone levels. Dose of rFSH in the obese group was greatly higher than that used in the underweight group ($P < 0.001$). Number of follicles in diameter 14–18 mm was higher ($P = 0.002$) in normal weight subjects compared to other subjects. Overweight subjects were with the lower implantation rate ($P < 0.001$), biochemical pregnancy rate per stimulation cycle ($P = 0.025$), as well as clinical pregnancy rate per transferred cycle ($P = 0.033$), compared to normal weight subjects. The miscarriage rate in the first trimester in total subjects was 2.2%. Total incidence of AEs was 47.8% with 20.4% as treatment-related. Severe AEs only occurred in 5 subjects (0.6%). Major recorded AEs included OHSS (11.8%), twin pregnancy (9.7%) and ectopic

pregnancy (1.5%). A total of 180 subjects (22.8%) had cycle cancelled due to risk of OHSS.

Limitations, reasons for caution: The sample size was still relatively small for estimation of BMI status in full PCOS population. Confounding factors other than BMI may have influenced the outcomes. Also no intervention was performed to assess the effect of BMI change on outcomes.

Wider implications of the findings: The overweight and obese population in Chinese PCOS patients seems to be in the lower range compared to the reported data in other populations, yet still significantly influence IVF/ICSI outcomes. Further studies are in need to explore whether interventions to normalize BMI reduce rFSH requirement and improve outcomes.

Trial registration number: None.

P-438 Localization of toll like receptors (TLRs) in human fallopian tube epithelial cells

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Study question: Are there any proteomic differences between expression and localization of the TLRs in fallopian tube epithelial cells?

Summary answer: There is a difference signature between expression and localization of the TLRs in fallopian tube epithelial cells.

What is known already: Infections of the female reproductive tract may contribute to infertility to a various extent depending on the site of inflammation, especially in fallopian tube. Histologically, fallopian tube epithelium is formed by a ciliated and secretory cells. The immune system related to epithelial cells of the FRT represents the first line of defence against sexually transmitted pathogens. Recognition of these pathogens is attributed to the family of Toll like receptors (TLR) as a major part of the innate immune system. This study clarifies the expression, function and localization of the TLRs in fallopian tube epithelial cells.

Study design, size, duration: The current study was approved by the Local Ethics Committee and informed written consent was obtained prior to the collection of tissue samples. Human fallopian tube tissues were collected from 5 cases undergoing tubal ligation. TLRs expression in fallopian tube ciliated and non- ciliated epithelial cells was investigated

Participants/materials, setting, methods: We firstly investigated TLRs localization in fallopian tube epithelial cells by immunostaining, surprisingly we found the intensity of staining was not equal in epithelial cells, then after primary cell culture of fallopian tube epithelial cells, ring cloning was used to isolate colonies of ciliated from non- ciliated epithelial cells and then the expression of TLR1–10 was examined by Quantitative real time PCR, and protein localization confirmed by immunostaining

Main results and the role of chance: We found TLR1–10 to be expressed in Fallopian tube epithelial cells, our studies revealed enriched localization of TLRs in Fallopian tube ciliated epithelial cells. We showed that TLRs expression in fallopian cells, with a higher level in the cilia cells versus non-ciliated cells ($P < 0.05$). Treatment fallopian tube epithelial cells with peptidoglycan, poly(I:C), flagellin, Loxoribine and CPG oligonucleotide resulted in secretion of IL-6 and IL-8.

Limitations, reasons for caution: Further experiments are needed to extend our understanding of function of the ciliated cells *in vivo*.

Wider implications of the findings: TLRs is localized in human Fallopian tube epithelial cells. Based on cyclical changes in ciliation and cell height of fallopian tube epithelial cells, under the influence of ovarian hormones, it seems immune system related to epithelial ciliated cells provide a safe environment for fertilization in the fallopian tubes

Trial registration number: This study is an experimental case controlled study.

P-439 Three dimensional ultrasound and hysteroscopy in diagnosing benign intrauterine lesions in infertile women

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Study question: The purpose of this retrospective study was to assess whether 3D ultrasound (3D US) could replace diagnostic hysteroscopy in infertile women prior assisted reproductive treatments (ART)

Summary answer: Our study suggest that 3D US is a safe alternative to hysteroscopy.

What is known already: Three dimensional ultrasound has recently been introduced into clinical practice. Whit this technology any desired place through an organ can be obtained. It has enabled the accurate, noninvasive, outpatient measurement of the follicular, ovarian and endometrial volumes feasible and the diagnosis of congenital uterine anomalies. In addition, 3D dimensional reconstruction of the uterine cavity is possible and can be analyzed without particular expertise in ultrasound diagnosis. At the same, it has very low inter-observer and intra observer variability for calculating endometrial volume and also was found to be highly reproducible for estimating ovarian and endometrial characteristics.

Study design, size, duration: A retrospective study during 2012–2013 was conducted comparing 3D transvaginal (TV) ultrasound evaluation and hysteroscopy at the "Momo' Fertilife" Private Centre for Reproductive Medicine, Bisceglie, Italy.

Participants/materials, setting, methods: The present study involved 519 women aged 21–48 years undergoing *in vitro* fertilization (IVF) techniques. The mean duration of infertility was 1–15 years. Transvaginal sonography was performed first in the follicular phase of the menstrual cycle. Assessment of morphological features, including uterine dimension, myometrial lesions, endometrial lesions and thickness, adnexal abnormalities was carried out. After the sonographic examination, hysteroscopy was performed within a day or two in the same menstrual cycle.

Main results and the role of chance: In 414 patients a normal uterine cavity was diagnosed by 3D US and hysteroscopy. A diagnosis of endocavitary uterine pathology was done in 105 cases and those patients were available for the analysis of diagnostic performance of the tests. Concordance between 3D ultrasound and endoscopy around was verified in all cases of endometrial polyps (50 cases- 47.6%), submucosal myomas (35 cases – 33.3%) uterine septa (15 cases – 14.28%). Differing results were found in 5 cases (4.76%) of endometritis diagnosed only by hysteroscopy. The diagnostic performance of 3D US examination versus hysteroscopy was expressed as accuracy, sensitivity and specificity. The sensitivity of 3D US for the diagnosis of intrauterine lesions was 87% vs. 100% of hysteroscopy. The specificity was 100% for both the tests. Positive and negative predictive values of three dimensional ultrasound and hysteroscopy were 100%, 99%, 100%, 1005 respectively.

Limitations, reasons for caution: In our study no correlation was found between endometrial measurements and pattern obtained by 3D US and histological diagnosis derived by endometrial biopsy during hysteroscopy. In fact, chronic endometritis is a subtle condition that is difficult to detect.

Wider implications of the findings: We suggest that 3D US should be the first clinical noninvasive diagnostic test in the investigation of the uterine cavity in infertile women. The total examination time for the patient is significantly reduced and all further investigations can be performed without the presence of the patient.

Trial registration number: No trial registration number

P-440 Preclinical data support the safety of egg precursor cell (EggPC) repositioning using a method designed to address poor response to controlled ovarian stimulation

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Study question: To test the safety of egg precursor cell (EggPC) repositioning in ovaries using the OvaPrime method in non-human primates, thus strengthening pre-clinical evidence supporting OvaPrime.
Summary answer: OvaPrime treatment is safe in non-human primates, thus supporting its investigation as a therapeutic approach for women with little or no egg reserve.
What is known already: Options are limited for women with poor ovarian response to controlled ovarian stimulation. OvaPrime is an innovative technique developed to address this unmet need by enhancing a woman's egg reserve through repositioning her own EggPCs in one/both ovaries to generate a good number of mature, high-quality eggs. This may improve live birth rate using autologous oocytes for women with poor ovarian response, diminished ovarian reserve, or primary ovarian insufficiency, among others. Preclinical studies demonstrated live births from EggPCs in mice and rats, and production of mature eggs from EggPCs in Rhesus macaques and immature human eggs in a xeno-transplanted model.
Study design, size, duration: A Good Laboratory Practices-level safety study in non-human primates. EggPCs were isolated from 1 ovary per animal and cryopreserved. Ovarian cortex tissue was mechanically and enzymatically digested, EggPCs isolated via antibody-mediated fluorescence-activated cell sorting and slow-frozen. Two weeks after ovarian removal, either 10% or 100% of the autologous EggPCs isolated from an individual were surgically reintroduced to the cortex of the contralateral ovary of the same animal via mid-line laparotomy, using a 25G needle.
Participants/materials, setting, methods: Overall, 18 female *Cynomolgus* monkeys (macaca Fascicularis) aged 3–4 years were evaluated. For up to 6 months after autologous EggPCs were reintroduced into the ovary, clinical and histological evaluations were conducted, including evaluation of the ovaries and reproductive organs, menstrual cycle assessment, body weight, hematology, serum chemistry, serum hormone analysis, and urinalysis. Sampling frequency varied by parameter (e.g., serum hormones sampled daily during certain phases of menstrual cycle, weight measured weekly, blood taken monthly).
Main results and the role of chance: In the 6 months after the OvaPrime treatment, no morbidity, mortality or treatment-related abnormalities were observed. Histology and clinical assessments were normal at 6 months post-transplantation. Specifically, no significant pre-to-post within-animal changes were observed for body weight, hematology, serum chemistry, or urinalysis parameters. All animals exhibited normal menstrual cycles following the EggPC reintroduction and there were no observed alterations in hormone levels related to menstrual cycles. The results demonstrate the safety of repositioning of up to 100% of EggPCs taken from a single ovary into the contralateral ovary using the OvaPrime method in non-human primates, adding to the evidence that it is safe and feasible and thus supporting future clinical evaluation of this treatment.
Limitations, reasons for caution: This was a safety study only; EggPC biologic activity after repositioning was limited to hormonal and menstrual cycle vs. estrus cycle continuation, and matings and pregnancy rates were not studied, so the effectiveness of the OvaPrime treatment (live birth rates) in non-human primates cannot be confirmed from this study.
Wider implications of the findings: Preclinical studies across multiple species have demonstrated the potential of EggPCs to increase the antral follicle pool. OvaPrime will be evaluated in the clinic as a potential therapeutic approach to address the significant unmet need in women with poor response to controlled ovarian stimulation by increasing the antral follicle pool.
Trial registration number: Not applicable.

P-441 MMPs and TIMPs gene expression regulates the human ovarian follicle, a microenvironment for oocyte differentiation

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Study question: Validation of metalloproteases (MMPs) and their inhibitors (TIMPs) as diagnostic biomarkers helping in prediction of oocyte quality and embryo viability in IVF cycles.
Summary answer: Our results suggest the deep involvement of MMPs and of their specific tissue inhibitors (TIMPs) in the proper maturation of the human oocyte.
What is known already: Follicular fluid, as well as granulosa and cumulus cells may affect follicle growth, oocyte maturation and competence acquiring. Unfortunately, valid biomarkers for oocyte quality estimation and, maybe, for *in vitro* fertilization success improvement have not been still identified. Metalloproteinases and their specific tissue inhibitors are implicated both in the homeostasis of the ovarian follicle and mainly in oocyte maturation, ovulation and corpus luteum organization. The estimation of oocyte quality for ART, still now unsatisfactorily limited to morphological parameters, may be implemented by gene expression analysis of these biomarker helping in the selection of the best oocyte to be injected.
Study design, size, duration: For this study, we have collected follicular fluids, granulosa and cumulus cells from fifty women who underwent techniques of assisted reproduction starting from January to October 2015. Gene expression analysis of MMP2, 9 and 11 and of TIMP1 and TIMP2 was carried out both in granulosa and in cumulus cells. Data obtained by gene expression analysis were also confirmed at protein level by western blot analysis and immunofluorescence techniques.
Participants/materials, setting, methods: To identify key molecules of the cross-talk between somatic cells and oocyte, we performed an *in silico* analysis of the human follicular fluid proteome. MMPs and TIMPs, resulting *central hubs* in the MetaCore generated network, were further evaluated by gene expression studies on granulosa and cumulus cells in a cohort of 50 women. Our results, confirmed by western blot and immunofluorescence, allowed us to validate the selected genes as potential biomarkers for oocyte quality prediction.
Main results and the role of chance: Our results revealed the deep involvement of MMPs, in particular gelatinases (MMP2 and MMP9), in the development and maturation of the oocyte, with a differential expression in granulosa compared to cumulus cells, thus highlighting the important role exerted by cumulus cells in line with the high specialization of this cell type. In fact, MMP2 and MMP9 expression was significantly increased in granulosa cells compared to cumulus cells, while mRNA levels of MMP11 were increased in cumulus cells. MMP11 seems to play an important role in oocytes physiology, confirming the hypothesis that this protein is involved not in follicular rupture, but in other tissue remodeling process, such as follicle atresia. In spite of this and according to their spectrum of action, MMPs modulation has to be carefully controlled in order to prevent deleterious effects on ovarian functions. Hence, in the same cell types the modulation of specific tissue inhibitors of metalloproteinases TIMP1 and TIMP2 results highly relevant. Indeed, while TIMP1 mRNA levels are comparable in these cells, TIMP2 expression level resulted significantly increased in cumulus compared to granulosa cells. Hence, the balancing in MMPs/TIMPs expression is crucial for oocyte development. MMPs and TIMPs may be precious pharmacological target to improve fertility.
Limitations, reasons for caution: Different parameters may influence ovarian response to hyperstimulation protocols as well as the outcome of IVF cycles, therefore larger study needs to be carried out in order to definitively validate these findings.
Wider implications of the findings: This combined *in-silico* approach and gene expression profile allowed us to identify MMPs and TIMPS as key factors whose expression could predict the oocyte release, its quality and fertilizability. Therefore our study, if replicated in a larger population, might contribute in improving oocyte selection and ART protocols in infertile women.
Trial registration number: Not applicable

P-442 Immature oocyte collection during ovarian tissue harvesting and cryopreservation. What is the yield and outcome?

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Study question: What is the yield of collecting immature oocytes *in vivo* and ex vivo as potential fertility preservation techniques ?
Summary answer: Although a high number of mature oocytes and embryo can be stored. The limited number of pregnancies present poor reproductive outcome.
What is known already: There are relatively few clinical options for fertility preservation and the data on their clinical outcome is limited. Retrieval and storage of immature oocytes followed by *in vitro* maturation (IVM) have been proposed as a possible supplementary approach to fertility preservation prior to cancer treatment.
Study design, size, duration: A retrospective cohort study of patients treated from 2007–2014 in single tertiary center in Israel. A total of 119 cancer patient were included in the study.
Participants/materials, setting, methods: Fifty seven patients underwent ovarian tissue cryopreservation (OTC) plus ex-vivo oocytes collection from the ovarian tissue (OTO-IVM). Twelve patients underwent in-vivo oocyte aspiration from both ovaries and IVM (OPU-IVM) and 50 patients underwent OTO-IVM and OPU-IVM. Main outcome measures were oocyte yield and outcome post thawing.
Main results and the role of chance: Obtaining oocytes using the combined technique of OTO IVM+OPU IVM yielded significantly more oocytes (11.87 ± 1.22 vs. 6.95 ± 0.83; $p < 0.001$), M2 oocytes (6.58 ± 0.7 vs. 2.47 ± 0.1; $p < 0.01$), maturation rate (57% vs. 35%; $p < 0.01$) and cryopreserved oocytes (6.00 ± 1.37 vs. 2.42 ± 0.49; $p < 0.01$), as compared to OTO IVM alone. Thirty two embryos from five patients were thawed (16 embryos from the OPU IVM and 16 embryos from OTO IVM+OPU IVM group). Thirteen embryos survived (81% survival rate) and were transferred in each group. One out of the 26 transfers yielded a pregnancy that resulted in a normal delivery of a healthy baby.
Limitations, reasons for caution: Low number of thawed embryos
Wider implications of the findings: Retrieval of immature oocytes is time consuming and relatively inefficient for the majority of the population. IVM maybe beneficial to young patients with high AFC as in PCOS and for those patients who are at high risk for reintroducing hemthological or other malignancies and cannot perform emergency IVF.
Trial registration number: 7222-09-SMC

P-443 Egg donors' characteristics and acceptance procedure according to the Israeli egg donation law

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Study question: To study the characteristics and mode of selection of the Israeli egg donors in accordance with the recent local egg donation (ED) law.
Summary answer: A third of the donors are academic, 44% are mothers of children. Their dropout rate due to the long and complicated acceptance procedure is 53.2%.
What is known already: ED involves ethical and moral issues. Few countries have settled an ED law, where donors' anonymity and rights are controlled. The law also defines the amount of compensation payment, where half of it is subsidized by the Ministry of Health in order to encourage ED. Computerized database of the Ministry controls the number of donations per donor and the matching between donor and recipient, ruling out the possibility of a relationship between both parties, and the eligibility of the donor to donate.
Study design, size, duration: For the past two years, ED has been performed in our Center. In the first interview general information is given and possible risks are explained. On her second visit signed informed consent is collected and an interview with a psychologist takes place. Following the approval of our Committee her candidacy is entered into the database of the Ministry of Health. So far 94 donors appeared for the first meeting with the physician and nurse coordinator.
Participants/materials, setting, methods: Donors were investigated for their past and family history, education and profession. The data from the psychologists and Confirmation Committee were examined and summarized. Objective data such as age, occupation, height, weight and proven fertility in the past were given to the recipient before her acceptance of the donor. The data were analyzed and evaluated.
Main results and the role of chance: Of the 94 applicants, 71% were unmarried, 13% divorced with children, 10% singles with children, and 8% married with children. Thirty one (33%) donors were students in the university. Sixty one (65%) were born in Israel and 34% were immigrants from Eastern Europe. Only 54 donors (57%) arrived for the second visit, 10% were not approved by the psychologist due to either an immature personality or psychiatric past history. The Committee did not approve 4 donors who were refused by the psychologist and an additional candidate who had a family history of Marfan syndrome. A total of 54 donors received final approval for the procedure, of them 5 (9.2%) decided not to donate. At the end of the procedure 38 donors (40.4%) performed ED. This complicated and long procedure resulted in the loss of many potential candidates. However, this thorough workup of the donors gives a good feeling to both, the medical staff and the recipients.
Limitations, reasons for caution: The size of the study group is limited, and so conclusions may not be well established. With a larger experience the law should be revised in order to simplify the procedure.
Wider implications of the findings: The procedure for acceptance of the donor is complicated and long. However, selection of donors is performed very carefully and the donor has several options to drop out of the project.
Trial registration number: NA

P-444 Uterine function and its relation with pregnancy complications and outcomes among childhood cancer survivors treated with radiotherapy involving the uterus

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Study question: Does uterine irradiation administered during childhood reduce uterine volume, and does it lead to an increased risk of pregnancy complications and adverse pregnancy outcomes?
Summary answer: Exposure to uterine irradiation during childhood significantly reduces adult uterine volume, and leads to an increased risk of pregnancy complications and adverse pregnancy outcomes.
What is known already: Different types of cancer treatment may impair female fertility. Radiotherapy (RT) administered during childhood appears to have a negative impact on uterine function, leading to reduced adult uterine volumes and diminished blood flow in the uterine arteries. However, studies investigating this topic had small RT-exposed study populations, and further comparison with other cancer treatments is necessary.
Study design, size, duration: The study is part of the DCOG LATER-VEVO study, a nationwide retrospective cohort study on female fertility in Dutch childhood cancer survivors (CCSs). Data collection took place between January 2008 and May 2014.
Participants/materials, setting, methods: Fifty-five CCSs who had been treated with radiotherapy involving the uterus (RT-exposed CCSs) were age- and parity-matched to two types of comparison groups: 110 CCSs not treated with any type of radiotherapy (non-RT-exposed CCSs) and 110 females from the general population (healthy controls). Uterine volume was

measured by three-dimensional transvaginal ultrasound. Information on pregnancy complications and pregnancy outcomes was obtained through a questionnaire.

Main results and the role of chance: Among the nulligravidous participants, median [interquartile range (IQR)] uterine volume was 41.1 (18.1–53.2) ml for RT-exposed CCSs, compared to 48.1 (35.7–61.8) and 61.3 (49.1–75.5) ml for non-RT-exposed CCSs and healthy controls, respectively. Results of the multivariate regression analyses show that, after correction for age and estradiol levels (as a measure of menopausal status), RT-exposed CCSs are at increased risk of having a reduced uterine volume (<34.9 ml) compared to both non-RT-exposed CCSs and healthy controls (OR=2.70 (95% CI 1.06–6.90) and OR=11.80 (3.29–42.30) respectively). Additionally, non-RT-exposed CCSs also appeared to be at increased risk of having smaller uterine volumes compared to healthy controls. This outcome was no longer significant after correction for alkylating agents dose-score. Furthermore, among gravidous participants, RT-exposed CCSs had an increased probability of ever having experienced a pregnancy complication and of ever having delivered pretermly (<37 weeks) compared to healthy controls (OR=12.70 (2.55–63.40) and OR=9.74 (1.49–63.60) respectively). Additionally, RT-exposed CCSs had an increased probability of delivering an infant with low birth weight (2500 gr) compared to both non-RT-exposed CCSs and healthy controls (OR=6.86 (1.08–43.75) and OR=15.66 (1.43–171.35) respectively).

Limitations, reasons for caution: For gravidous participants, we had no information on uterine volume prior to pregnancy. This severely hampered our ability to investigate the effect of reduced uterine volume on risk of pregnancy complications and adverse pregnancy outcomes.

Wider implications of the findings: CCSs exposed to uterine irradiation should be counselled early about increased risks of impaired uterine function, pregnancy complications and adverse pregnancy outcomes. Furthermore, close obstetric monitoring of CCSs exposed to uterine irradiation is advised in case of pregnancy. Further research is necessary to investigate the chemotherapy-related effect on uterine volume.

Trial registration number: NTR2922

P-445 Freeze all policy in cases of high risk of ovarian hyperstimulation syndrome does not impair cumulative pregnancy rates in a center with slow freezing program

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Study question: Should our frozen pregnancy rate be a limitation for a freeze all policy in cases of high risk of ovarian hyperstimulation syndrome (OHS)?

Summary answer: With 11.6% difference in pregnancy rate between fresh and frozen embryos transfers, no harm in cumulative pregnancy rate was observed with this policy.

What is known already: To avoid OHS, a differed embryo transfer has been proposed as a very effective security tool. Nevertheless, it seems difficult to settle without a high effective cryopreservation program, because it may affect the pregnancy expectations of patients.

As embryo vitrification seems to be the more effective embryo freezing technique, centers with slow freezing cleavage embryo program and lower cryotransfer pregnancy rates may be reluctant to switch to a freezing all attitude. A correct patients selection of high risk of OHS and good prognosis women should offer equivalent outcome in terms of cumulative pregnancy rate in a hyperstimulation free milieu.

Study design, size, duration: In a retrospective case control study, 50 high-risk OHS women defined as patients with more than 15 oocytes obtained, final Estradiol (E2) >3000 pg/nl or previous history of OHS, were included. We selected 25 cases and 25 controls, completing all embryo transfers from the study stimulation cycle without pregnancy or who got pregnant after one of the transfers. Patients where selected between January 2013 and June 2014. Primary end point was cumulative pregnancy rate.

Participants/materials, setting, methods: First completed 25 cases of OHS risk patients who underwent freezing all policy in 12 de Octubre Hospital were included. One control per case was selected between 2014 cycles considering that no difference in cryopreservation program or in embryo culture or transfer technique had been introduced in our unit meanwhile. They all had similar characteristics regarding ovarian reserve parameters, previous cycle results or OHS risk factors.

Main results and the role of chance: Cumulative pregnancy rates were the same in both groups: 64% (16 pregnant patients per group, $p > 0.05$) even if 7 out of the 16 pregnancies (43.75%) occurred in the fresh transfer in the control group. No severe OHS was observed but 3 women in the control group had to be controlled for mild OHS. No difference could either be found in miscarriage rate (6.2% in both groups).

Cycles characteristics were similar in both groups: final E2 of 3662.6 ± 16.10 in the cases vs. 3072.8 ± 1074.69 in the control group ($p > 0.05$); follicles 19.5 ±5.42 and 19.8 ± 4.37 ($p < 0.05$), oocytes retrieved 14.2 ± 4 and 16.8 ±3.53 ($p < 0.05$); embryos available 7.4 ± 3.25 and 7.7 ± 2.51 ($p < 0.05$) with 2.9 ± 2.43 and 2.8 ± 2.41 good quality embryos respectively ($p < 0.05$).

Those were young patients (33.8 ± 3.89 years in case group and 32.4 ± 5.19 in control group; $p < 0.05$); good antral folicular count (19.7 ±9.71 and 16.8 ± 5.26; $p < 0.05$) and normal FSH basal level. Clinical pregnancy rate per thawed transfer embryo is not comparable to general IVF patient population (37.2% in cases; 19.24% in controls and 17.8% in general population).

Limitations, reasons for caution: The retrospective design is an important study limitation, although differences were favorable to control group.

Characteristics of the selected population: young women with 40% of polycystic ovarian syndrome (in both cases and control group) should be considered before extrapolating data, as well as the type of cryopreservation policy and protocol.

Wider implications of the findings: This example may help other center with slow freezing technique or sub-optimal pregnancy rate in frozen/thawed cycles to undergo a freezing all policy in high-risk OHS patients. These selected patients have an especially favorable prognosis with a high cumulative pregnancy rate with no impairment in pregnancy chances and safer conditions.

Trial registration number: None

P-446 Immature myeloid cells in reproductive system versus tumor angiogenesis

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Study question: We sought to determine whether ovarian stimulation and placental development leads to an influx of proangiogenic immature myeloid cells as observed in tumors.

Summary answer: IMC subpopulations diverge in tumor versus reproductive tissues, favoring monocytic IMCs in the former and granulocytic IMCs in the latter.

What is known already: In close resemblance to other inflammatory states, gonadotropin stimulation is accompanied by influx of inflammatory cells such as neutrophils and monocyte-derived effector cells, into the ovarian stroma. Ovarian follicular development and corpura lutea formation are examples of the few physiologic events in which the formation of new blood vessels form existing ones, i.e., angiogenesis takes place. We have previously shown that bone marrow derived immature myeloid cells (IMCs) promote angiogenesis in both health (placentas) and disease (malignant tumors) and thus express similar proangiogenic genes. Nevertheless, the unique properties of IMCs populating these physiologic- versus malignant tissues have not been explored.

Study design, size, duration: Animal experiment, using 4–5 week old C57Bl6J-Cx3CR1$^{GFP/+}$ transgenic mice, in which granulocytic (Ly6G^{+}) and monocytic (Ly6C^{+}) IMCs can be defined by flow cytometry. We analyzed placentas from timed pregnancies and tumor tissues from B16-melanomas that were implanted subcutaneously. For ovarian hyperstimulation we treated mice with 20U pregnant mare serum gonadotropins (PMSG) for 2 days. On day 3 human chorionic gonadotropin (HCG) (5U) was injected to induce ovulation. Control unstimulated mice were treated with sham injections.

Participants/materials, setting, methods: Harvested tissues were weighed and enzymatically digested. Single cell suspensions were immunostained using fluorescently labeled anti-CD11b, Gr-1, CD11c, major histocompatibility II (MHCII), CD45, Ly6G, and Ly6C and analyzed by flow cytometry. For global gene expression, IMCs were isolated by flow cytometry, RNA prepared and

analyzed by Affymetrix gene microarrays. Validation of single gene expression was performed by qPCR.

Main results and the role of chance: Analysis of the subpopulations of IMCs revealed a significant enrichment (over 2-fold, $P < 0.01$) of the $Ly6G^{med}/Ly6C^{high}$ monocytic IMC fraction in tumor derived $CD45^{+}$ hematopoietic cells compared to placenta, paralleled by a concomitant, more than 2-fold decrease ($P < 0.01$) of the $Ly6G^{high}/Ly6C^{med}$ granulocytic IMC subpopulation. In gonadotropin stimulated ovaries we observed a ~2.5 fold increase in Ly6Ghigh/$Ly6C^{med}$ granulocytic IMCs compared to unstimulated controls. Tumor derived- and gonadotropin stimulated ovaries derived $Ly6G^{med}/Ly6C^{high}$ IMCs expressed low levels of Cx3CR1 compared to the same cell population in placentas and unstimulated ovaries. Decreased expression of Cx3CR1 within IMCs has been shown to delineate a cellular population that actively contributes to tumor progression.

We next assessed the global transcriptional signature of tumor derived IMCs (T-IMCs) compared to placental IMCs (P-IMCs). Analysis of the top overexpressed genes in T-IMCs revealed several key players in tumor angiogenesis including Sema3a, and matrix metalloproteinases such as Mmp2, Mmp3, Mmp13, and Mmp14, as well genes that are involved in cancer progression and cell proliferation. Of note, various genes that were up-regulated in P-IMCs were shown to play a role in reproductive tissue angiogenesis, including Serpine1, Arg1, and Flt1.

Limitations, reasons for caution: This is an animal experiment and its findings need to be further validated also in humans.

Wider implications of the findings: IMC subpopulations diverge in tumor versus reproductive tissues, favoring monocytic IMCs in the former and granulocytic IMCs in the latter. This divergence is associated with unique expression of proangiogenic genes. Selective targeting of these genes may thus be further investigated as selective angiogenic therapies for cancer, placental disease, and ovarian-hyperstimulation.

Trial registration number: NA

P-447 Current but not former use of combined contraception is associated with glucose metabolism disorders in premenopausal women: a prospective population-based cohort study

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Study question: Is the former or current use of combined hormonal (CHC) and progestin-only (POC) contraceptives associated with abnormal glucose metabolism in premenopausal women?

Summary answer: Former CHC or former/current POC use was not associated with prediabetes/type 2 diabetes (T2DM), but the association was significant in current CHC users.

What is known already: Combined contraceptive use has been associated with impaired glucose tolerance (IGT) and increased insulin resistance, which are risk factors for prediabetes and T2DM. To date there are only few population-based studies with large enough population and with adequate adjustments. The results concerning hormonal contraceptive use and prediabetes/T2DM are inconsistent and no clear association between them has been demonstrated in general populations, but some prospective population-based studies have revealed an increased risk in current CHC users.

Study design, size, duration: In a prospective follow-up of a large national birth cohort ($n = 5889$) the women were clinically examined at the age of 46 and asked for their former and current use of hormonal contraceptives. The glucose metabolism indices were compared between current CHC ($n = 194$) or POC ($n = 1090$) and current non-hormonal contraceptive users ($n = 1204$), and on the other hand between former CHC ($n = 1884$) or POC ($n = 72$) users and women with no history of hormonal contraceptive use ($n = 358$).

Participants/materials, setting, methods: At age 46, a questionnaire was sent to 5123 women. Of them, 3708 (72.4%) answered, 3280 (64.0%) participated in clinical examinations and 2780 in a 2-h oral glucose tolerance test (OGTT). Prediabetes (impaired fasting glucose, IFG and/or IGT) and newly diagnosed T2DM (nT2DM) were diagnosed by OGTT. Diagnosis for previously diagnosed T2DM (pT2DM) was assessed from postal questionnaires and confirmed from hospital discharge and national drug registers.

Main results and the role of chance: Current CHC, POC and non-hormonal contraceptive users as well as former CHC and POC users and women who had never used hormonal contraception did not differ regarding BMI and waist circumference.

Current CHC use was significantly associated with prediabetes (odds ratio, OR: 2.1, 95% confidence interval, 95% CI: 1.3–3.4), nT2DM (OR: 3.4, 95% CI: 1.4–10.1), prediabetes/nT2DM (OR: 2.2, 95% CI: 1.5–3.5) and prediabetes/nT2DM/pT2DM (OR: 1.9, 95% CI: 1.3–2.9), but not with pT2DM compared with use of non-hormonal contraception.

Current POC use was not significantly associated with pre-diabetes, nT2DM or pT2DM compared with use of non-hormonal contraception.

Compared with current POC use, current CHC use was significantly associated with prediabetes (OR: 2.0 95% CI: 1.2–3.1) and prediabetes/nT2DM (OR: 2.0, 95% CI: 1.3–3.1), but not with pT2DM. Former CHC use was not significantly associated with prediabetes or nT2DM/pT2DM compared with former POC use or never use of hormonal contraception.

Former use of POC was not associated with prediabetes or pT2DM/nT2DM compared with never use of hormonal contraception.

The results did not change after adjustment for socio-economical status, alcohol consumption, smoking, number of deliveries and use of cholesterol lowering medication.

Limitations, reasons for caution: Use of hormonal and non-hormonal contraception was based on self- reporting which may have led to information bias. We excluded women reporting CHC use at any time of their life from the group of former POC users, which could have distorted the results.

Wider implications of the findings: Current CHC use in premenopause associates with a significant risk for prediabetes and T2DM, supporting active screening for these disorders in premenopausal CHC users. These findings also raise the question of whether it is advisable to recommend CHC use in older premenopausal women and in women with adverse metabolic profile.

Trial registration number: NA

P-448 CBL gene is a central gene overexpressed in cumulus cells of obese Polycystic Ovary Syndrome (PCOS) women without clinical insulin resistance compared to non-obese PCOS

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Study question: Which genes of the insulin pathway are relevant in cumulus cells of obese PCOS women when undergoing to an IVF cycle?

Summary answer: The gene CBL is overexpressed and acts as central gene of insulin signaling pathway in the cumulus cells of obese PCOS women.

What is known already: Insulin plays a central role on PCOS women and can be responsible for worst IVF outcomes on those patients. The precise mechanism of insulin action on cumulus cells and the consequences to oocyte maturation is not completely elucidated in obese or even in non-obese PCOS patients. PCOS women have oocytes of poorer quality even without clinical evidence of insulin resistance. The gene expression profiles from insulin pathway in cumulus cells could provide new insights, mainly in obese patients, where hyperinsulinemia should be deleterious and may explain the

worst IVF outcomes. New therapy strategies would be possibly offered to those patients.

Study design, size, duration: Cross-sectional study to evaluate gene expression at the insulin pathway in *cumulus* cells of patients submitted to IVF treatment from January 2013 to October 2014. Fifteen PCOS patients according to Rotterdam criteria were subdivided in non-obese (n = 9, BMI <25, Control-group) and obese (n = 6, BMI >30, Obese-group). Both groups demonstrated a normal insulin resistance index according to the homeostasis model assessment insulin resistance (HOMA-IR).

Participants/materials, setting, methods: PCOS infertile patients were submitted to IVF with standard ovarian stimulation protocol, oocytes were recovered to IVF and cumulus cells were removed for gene expression analysis. RNA purification was carried out and quantitative PCR array analysis of gene expression profile (RT^2 Profiler™ PCR Array Human Insulin Signaling Pathway- PAHS-030ZC-Qiagen, USA) was done according with manufacturer instructions. We considered genes up- or down-regulated in obese-group compared to control-group, those presenting fold-change ≥3 or ≤3 and $p < 0.05$.

Main results and the role of chance: Among the 84 genes of insulin pathway analyzed, nine genes were statistically significant overexpressed in obese patients compared to non-obese. There were no down-regulated genes in obese-group compared to controls. The up-regulated genes were BCL2L1 (fold=4.7; p = 0.021), BRAF (fold=3.8; p = 0.031), DOK1 (fold=4.6; p = 0.040), FBP1 (fold=5.3; p = 0.011), FRS2 (fold=4.1; p = 0.044), PCK2 (fold=4.4; p = 0.041), RPS6KA1 (fold 4.2; p = 0.044), SORBS1 (fold=3.6; p = 0.014) and CBL (fold=6.2; p = 0.019). The mentioned genes are mainly involved on glucose uptake and cell proliferation, regulation of insulin sensitivity and promoting gluconeogenesis, diminishing available pyruvate. All actins could damage the energetic balance in early stages of embryo development. The bioinformatics tool (SABiosciences – GNCPro™ –Qiagen, USA) was used to evaluate the network and interaction among up-regulated genes. That analysis showed that 75% of the overexpressed genes have a strong relationship to CBL gene. CBL is a gene involved in cell signaling and protein ubiquitination, which inhibits intracellular signal transduction by targeting some tyrosine kinases. There is growing evidence that CBL acts inhibiting systemic insulin resistance promoted by macrophage pro-inflammatory cytokines, which are secreted in adiposity tissue of obese women.

Limitations, reasons for caution: A small sample size was included to screen the 84 genes. However, the sample power was calculated and higher than 80%. Although insulin resistance was not clearly demonstrated, insulin may play different effects in obese patients. Also ovarian stimulation might produce damages in the cumulus cells and oocyte competence.

Wider implications of the findings: CBL is a central gene to the perfect cumulus cells signaling function. In obese PCOS patients, where insulin resistance leads to hiperinsulinemia and may selective overexpress the CBL activity, which could promote detrimental effects on the follicle development.

Trial registration number: Not applicable

P-449 GnRH agonist triggering reduces pain symptoms during IVF/ICSI cycles

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Study question: To assess the dynamic of pain symptoms during IVF/ICSI cycles when final oocyte maturation is triggered by GnRH-agonist (GnRH-a) or hCG.

Summary answer: Using GnRHa for triggering ovulation limits the progression of pain symptoms following IVF/ICSI as compared to hCG triggering.

What is known already: Timely administration of GnRH-a has been shown to induce a surge of endogenous LH and FSH and trigger ovulation. The advent of antagonist COS protocols allows to use GnRH-a trigger, although endometrial alterations make deferring ET preferable. The shorter duration of LH effects on the growing follicles as compared to the long-lasting stimulation exerted by hCG reduces or suppresses the risk of OHSS. It therefore plausible that GnRH-a trigger might reduce the pain aggravation normally encountered in IVF/ICSI after ovulation induction and oocyte retrieval.

Study design, size, duration: This is a retrospective observational cohort study nested in a prospective published cohort (Santulli et al., 2015) conducted between 01/01/2014 and 31/12/2014 in a tertiary care university hospital. A total of 122 cycles from patients who underwent IVF or ICSI programs were analysed. Patients received an oral contraceptive synchronization treatment followed by ovarian stimulation with FSH and oocyte triggering with an injection of GnRHa or rhCG. Only non-pregnant women were retained for this study.

Participants/materials, setting, methods: Women were allocated to two groups: GnRHa triggering with a scheduled deferred embryo-transfer (n = 70) or hCG triggering with a fresh embryo transfer (n = 57). Pelvic pain scores were evaluated using a visual analogue scale. Total VAS score was defined as the sum of VAS scores of the different painful symptoms. Two evaluations were performed: during oral contraceptive synchronization treatment before ovarian stimulation and three weeks post-retrieval. Univariate and multivariate logistic regression models were conducted.

Main results and the role of chance: VAS average values for each symptom and for Total VAS score were comparable except for dysmenorrhea at final evaluation which was lower in "GnRHa triggering" group (4.81 ± 3.23 and 3.52 ± 3.23, p = 0.046). For both groups, pain increased during ART procedure. Trends for Total VAS change, calculated by subtracting the final VAS score evaluation from that at synchronization evaluation, revealed a significant lower pain increase in "GnRH-agonist triggering" group compared to "hCG triggering" group (3.77 ± 7.73 and 6.50 ± 6.57, $p < 0.05$, respectively). After multivariate logistic regression, GnRH-agonist triggering was associated with lower pain increase during the ART as compared to "hCG triggering group" (OR=0.31, IC95% 0.13–0.71, p = 0.006).

Limitations, reasons for caution: For comparing the dynamic of pain without biases from a developing pregnancy only non-pregnant women in "hCG triggering" group were analysed. Yet, we cannot rule out that selecting the non-pregnant sub-group of women did not introduce a bias of its own.

Wider implications of the findings: Finding that GnRH-a triggering is associated with less pain makes this the primary ART strategy for any woman fearing pain or at increased risk of pain, as for example in case of endometriosis. GnRH-a triggering is therefore a sound low pain option when ART related pain is best avoided.

Trial registration number: 0

P-450 Single human spermatozoon freezing technique for cryptozoospermia or non-obstructive azoospermia patients

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Study question: Is it possible to freeze a single spermatozoon and keep a high recovery rate to improve the clinical outcome of cryptozoospermia or non-obstructive azoospermia patients?

Summary answer: Our data indicates that it is highly possible to freeze a single spermatozoon with a high survival rate of about 80%.

What is known already: Clinical outcome of non-obstructive azoospermia or cryptozoospermia has increased after ICSI when using fresh spermatozoa. When oocyte collection cannot be performed on the same day of the Micro-TESE or in the case of cryptozoospermia, cryopreservation of collected spermatozoa becomes crucial. However, conventional freezing procedures are not appropriate for very low numbers of spermatozoa with poor motility.

Study design, size, duration: We performed a retrospective analysis of the clinical outcome of 79 ICSI cycles using our novel cryopreservation procedures for ejaculated spermatozoa from 27 cases of cryptozoospermia in 52 cycles and testicular spermatozoa from 20 cases of non-obstructive azoospermia in 27 cycles from January, 2012 to December, 2014.

Participants/materials, setting, methods: This study dealt with 27 men with cryptozoospermia and 20 men with non-obstructive azoospermia. Moving spermatozoa were carefully aspirated into a pipette one by one and put into a micro-drop of medium. About 2 µl of freezing medium was put on the tip of the CRYOTOP. 1–10 sperms were aspirated into an injection pipette and inserted into the medium and left in liquid nitrogen vapor for 2 min before being stirred in liquid nitrogen.

Main results and the role of chance: Five healthy babies (5 ongoing) from 47 patients were born following this freezing method. Clinical outcome is listed in the following table.

Clinical outcomes	Cryptozoospermia	Non-obstructive azoospermia
No. of patients	27	20
No. of treatment cycles	52	27
No. of sperm frozen [per Cryotop®]	543	248
Sperm recovery rate after thawing (%)	81.03 (440/543)	78.23 (194/248)
Motile sperm rate after thawing (%)	57.27 (252/440)	40.72 (79/194)
No. of ICSI oocytes	215	98
Fertilization rate (%)	33.02 (71/215)	37.76 (37/98)
No. of embryos cultured for 5 days	42	19
Blastocyst rate (%)	45.24 (19/42)	36.84 (7/19)
No. of cycles transferred embryos	28	13
Clinical pregnancy rate (%)	28.57 (8/28)	38.46 (5/13)
Miscarriage rate (%)	25.00 (2/8)	20.00 (1/5)
No. of ongoing pregnancies	2	3
No. of births	4	1

Limitations, reasons for caution: This freezing procedure needs a special apparatus (Inverted microscope equipped with a micromanipulator) and requires a high level of technique for gathering and expelling 1–10 spermatozoa correctly and swiftly. So this procedure can only be performed by specialists, precious sperm can easily die with poor technique.

Wider implications of the findings: Once this procedure is mastered, the clinical outcome of severe male infertility is definitely improved. Especially in the treatment of azoospermia, precious spermatozoa surgically collected from epididymis or testicles can be cryopreserved to avoid repeating a biopsy. This procedure can lessen the patients' physical, mental and financial burdens.

Trial registration number: None.

P-451 Activin A mediates cumulus expansion by inducing hyaluronan accumulation and versican expression through Smad signaling pathways in human granulosa cells

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Study question: What are the effects of activin A on the two key factors for cumulus expansion (hyaluronan and versican) in human granulosa cells and the molecular mechanisms?

Summary answer: Activin A induces hyaluronan synthase 2 (HAS2) and versican expression as well as hyaluronan accumulation through Smad2/3-Smad4 signaling pathways in human granulosa cells.

What is known already: Ovarian cumulus expansion is critical for ovulation. Hyaluronan is the fundamental component of expanded cumulus in preovulatory follicle, and HAS2 is its major synthase. Moreover, appropriate interaction between versican and hyaluronan is necessary for stabilization of expanded cumulus. Knockout studies show that activin and other TGFβ superfamily members (GDF9, BMP15) are vital for ovulation and female fertility in mice. However, the effects of activin A on cumulus expansion are species dependent. To date, a few studies show that activin A increases Has2 expression in mouse cumulus cells, meanwhile the effects of TGFβ on versican were determined only in cancer cells.

Study design, size, duration: We used SVOG cells and primary human granulosa cells for *in vitro* experiments. SVOG cells were immortalized human granulosa cells originally obtained from women undergoing *in vitro* fertilization (IVF). Primary human granulosa cells were obtained from patients undergoing IVF. Both cells were cultured with activin A up to 24 h to investigate its effects on HAS2 and versican expression and hyaluronan production, SVOG cells were cultured up to 12 h to investigate the signaling pathways.

Participants/materials, setting, methods: Levels of mRNA and protein were detected by RT-qPCR and western blotting, respectively. The accumulation levels of hyaluronan were examined by enzyme-linked immunosorbent assay (ELISA). Specific siRNA approaches were applied to knock down endogenous Smad2, Smad3 and Smad4 in SVOG cells.

Main results and the role of chance: In immortalized human granulosa cells, activin A upregulated both HAS2 and versican levels in time dependent manners ($p < 0.05$, respectively). Meanwhile, treatment of activin A induced hyaluronan production ($p < 0.05$). The inductive effects of activin A on versican, HAS2 and hyaluronan production in SVOG cells were abolished by TGF-type I receptor (TβRI) inhibitor. Moreover, activin A activated both Smad2 and Smad3 phosphorylation. Knocking down of Smad2 or Smad3 alone partially attenuated the activin A-induced HAS2 and versican levels in mRNA and protein levels, suggesting that Smad2 and Smad3 were both required for the upregulation of HAS2 and versican by activin A in SVOG cells. In addition, inhibition of Smad4, the essential common Smad for activin/TGF-β signaling pathway, completely abolished the activin A-induced HAS2 and versican expression in SVOG cells.

To better simulate the physiological situation, we also used primary cultured cells. In primary human granulosa cells, activin A increased HAS2 and versican expression ($p < 0.05$ and $p < 0.01$, respectively). Also, activin A induced hyaluronan accumulation in the culture medium ($p < 0.05$), this effect was subsequently blocked by TβRI inhibitor. Taken together, our data showed that activin A upregulated versican and HAS2 expression and induced hyaluronan production through Smad2/3-Smad4 signaling pathways in human granulosa cells.

Limitations, reasons for caution: We conducted only *in vitro* experiments, without further confirmation of the effect of activin A on hyaluronan and versican levels *in vivo*. Because this part of study was completed in UBC, SVOG cells were kindly provided by Prof. Leung. I intend to add animal study in the future in China.

Wider implications of the findings: For the first time, our data indicate that in human granulosa cells, activin A induces versican expression as well as hyaluronan accumulation via Smad signaling pathways.

This gives a better understanding on the subject of the effect of TGFβ superfamily members on cumulus expansion and ovulation, especially in human being.

Trial registration number: This is a basic science trial.

P-452 Similar egg recipient pregnancy rates are obtained following donation from egg-share providers or altruistic egg providers

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Study question: Do oocytes donated from a sub-fertile woman as an egg-share provider (ESP) produce lower pregnancy rates than those donated from a healthy altruistic egg provider (AEP)?

Summary answer: Our results confirm similar recipient pregnancy rates following D3/D5 embryo transfer regardless of the source of the donor eggs.

What is known already: Few large studies have addressed this issue, principally due to the low numbers of AEP in the UK. A report from Oyesanya et al. (2009) suggested similar pregnancy rates could be obtained but the number in each group were low. Most UK studies have compared outcomes between the ESP and her recipient. These have suggested similar pregnancy rates and also that a lower age of the ESP and success in the ESP are predictive of success (Ahuja et al., 1998).

Study design, size, duration: A retrospective study over 3 years between January 2012 and December 2014, involving all matched egg share providers with their recipients, and all matched altruistic egg share providers and their recipients. We excluded all egg shared providers donating to their female partners in same sex relationships.

Participants/materials, setting, methods: In 3 years there were 211 matched pairs of egg providers with recipients. The matching was performed by a nurse taking into account the physical characteristics of the recipient/provider. These details and a personal written profile from the provider were sent to the recipient who could then accept or decline. 93 pairs were in the egg share group and 118 pairs involving altruistic egg donation.

Main results and the role of chance: During the 3 years there was an increase in altruistic egg providers from 15 in 2012 to 57 in 2014. The number of egg share providers remained the same at ~30/year.

The ages of the recipients were similar in the 2 groups, but the AEP were significantly younger than the ESP ($p < 0.001$). The altruistic egg recipients (AER) received significantly more eggs than the egg share recipients (ESR) 11 compared with 7 ($p < 0.001$): created significantly more embryos 7 compared with 5 ($p < 0.001$); and were significantly more likely to have embryos for cryopreservation 71% vs. 46% ($p < 0.001$)

However the pregnancy rates/ET were similar for all 3 groups. For D3 transfer 25%; 26%; 31% and for D5 transfer 61%; 53% and 56% for the ESR, AER, and ESP, respectively.

The age of the provider, her serum AMH and whether or not she became pregnant, did not influence the likelihood of pregnancy in the recipient.

Limitations, reasons for caution: The study involves a reasonable number in each group but larger numbers would always be beneficial

The results of the frozen embryo transfer cycles are not available. This may indicate a higher cumulative pregnancy rate in the AER group due to the greater number of women with embryos cryopreserved.

Wider implications of the findings: This study confirms that ESP should still be used in an oocyte donation programme as they do not reduce their own chance of a pregnancy through their donation, and they provide the same chance of a pregnancy to the recipient, as those receiving eggs from an AEP.

Trial registration number: NA.

P-453 Analysis of cumulative Live Birth Rate (cLBR) after up to five IVF/ICSI treatments in relationship to the number of the complete cycle

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Study question: What is the relationship between the number of complete IVF/ICSI treatment and the cLBR considering previous completed treatments?

Summary answer: The chance of cLBR is higher at the first attempt, and there is no statistically significant decrease from the second attempt up to the fifth.

What is known already: Despite the growing success rates of IVF treatments during the last decade many couples have to undergo several cycles before obtaining live birth. Surely, it is important to link the success and so the LBR to the age of the woman, but this information is not useful for patients who underwent previous treatments or those who intend to undergo multiple cycles.

Some authors assumed as the first cycle, the first one performed in their centres, others showed the results/single episode of transfer without investigating the real chance of the completed cycle (fresh + frozen) after a certain number of treatments.

Study design, size, duration: This retrospective multicentric cohort study analyzed 4200 complete IVF cycles conducted between May 2009 and June 2013. One complete cycle was defined as the transfer of all fresh and frozen embryos resulting from one ovarian stimulation. Complete cycle data were stratified according to the number of the completed cycle (from the first to the fifth treatment) and the woman's age. The mean woman age was 35.8 ± 4.1.

Participants/materials, setting, methods: Participants were between 18 and 43 years old undergoing oocytes retrieval at one of the IVF centres. We included cycles with at least one retrieved oocyte. We discussed the relationship between the number of treatment and clinical outcome in terms of cLBR and then, thanks to the high number of cycles analyzed, we stratified and evaluated this relationship in the different age groups (Group 1: ≤34 years, Group 2: 35–38 years, Group 3: 39–43 years).

Main results and the role of chance: Out of 4200 complete IVF cycle, 1210 resulted in a live birth (28.8%). The overall analysis showed the following cLBR/cycle: 31.8% at the first treatment, 26.7% at the second one, 23.1% at the third one, 25.0% at the fourth one, 18.2 at the fifth treatment.

Statistical analysis shows the first treatment with the highest success rate ($p < 0.05$). From the second treatment up to the fifth no significant differences were found ($P = 0.143$).

cLBR was 36.4, 30.3, and 17.3% in Group1, Group2 and Group3, respectively. The analysis of groups according to the patient age and number of complete treatment showed that in Group 1 the cLBR/cycle is significantly higher at the first treatment ($p < 0.05$), whereas by the second to the fifth one there is no statistically significant difference between the rates ($P = 0.065$) (Group 1: 38.7%, 35.3%, 27.8%, 31.3%, 12% respectively). There is no statistically significant difference by the first treatment until the last one in Groups 2 and 3 (Group 2: 32.8%, 27.3%, 27.2% 28.2%, 29.4%; Group3:18.5%, 17.4%, 14.1%, 19.4%, 8.9% respectively).

Limitations, reasons for caution: As this is a retrospective study, the information obtained is based on previously recorded data. Consequently, certain informations that could influence the outcome, such as total gonadotropin consumption, BMI, markers of ovarian reserve and others, are not in the dataset.

Wider implications of the findings: Our data shows as cLBR is highest at the first attempt while by the second to the fifth no significant decrease is recorded. It could be helpful for linking the number of treatment to likelihood of cLBR suggesting a substantial positive potential of further treatment cycles, when not physiologically contraindicated.

Trial registration number: Not a clinical trial.

P-454 A higher risk of the metabolic syndrome in subfertile women

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Study question: Is the incidence of the metabolic syndrome higher in subfertile women and is it related to the cause of their subfertility?

Summary answer: The prevalence of the metabolic syndrome is higher in subfertile women, but is not related to the cause of subfertility.

What is known already: The metabolic syndrome is a well-known risk factor for cardiovascular disease. Subfertile women may have a higher risk of cardiovascular related problems. For instance, the risk of developing preeclampsia increases in women with a reduced ovarian reserve who receive an IVF treatment. Furthermore obesity, a parameter of the metabolic syndrome, is associated with reduced fecundity in subfertile ovulatory women. Several studies show subfertility is associated with an elevated risk of maternal obstetric complications. Whether this is the result of the used artificial reproductive technique (ART) procedures or related to the health of the subfertile woman is still an ongoing debate.

Study design, size, duration: We performed an interim-analysis of an ongoing prospective cohort study.

Participants/materials, setting, methods: Subfertile women who visited the Maastricht University Medical Center ($n = 41$) were included. The control group consisted of participants with a history of an uneventful spontaneous pregnancy ($n = 13$). We collected clinical measurements, blood and urine samples to determine the variables necessary to define the metabolic syndrome. This definition was met when a participant fulfilled the criteria of the World Health Organisation (WHO), the Third Adult Treatment Panel (ATP) III or the International Diabetes Federation (IDF).

Main results and the role of chance: The incidence of the metabolic syndrome in the overall study group of subfertile women was 13.2% (5/38), 5.4% (2/37) and 13.8% (4/29), according to the WHO-, ATP III- and IDF-criteria respectively compared to 0% (0/13, 0/13, 0/13) in the control group; OR 1.40 [95% CI 1.16–1.67], OR 1.37 [95% CI 1.15–1.63] and OR 1.52 [95% CI 1.21–1.91]. Nineteen women were diagnosed with an unexplained subfertility. In this subgroup of unexplained subfertility an incidence of 0% for all definitions was found. A significant higher diastolic blood pressure and waist-to-hip ratio was found compared to the control group, respectively from 67.5 (55–90) to 62.0 (53–79) mmHg and 0.81 (0.70–1.01) to 0.75 (0.69–0.87). In the study subgroup with unexplained subfertility a statistical significant higher waist-to-hip ratio, blood pressure and mean arterial pressure (MAP) were found compared to the control group (p-value <0.05).

Limitations, reasons for caution: We performed an interim-analysis in an ongoing study and therefore the presented sample sizes are still small. Our clinic is not performing fertility work-up and treatment in women with a body mass index >35 kg/m^2.

Wider implications of the findings: The results show that it can be beneficial to screen all subfertile women for the metabolic syndrome. Lifestyle interventions or medical treatment of the metabolic syndrome may result in a higher chance of an uneventful pregnancy.

Trial registration number: Clinicaltrials.gov, NCT02136472.

P-455 miRNAs expression profile in human oocytes from women undergoing different IVF stimulation protocols

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Study question: Which is the role of IVF stimulation protocols in modulating oocyte miRNome?
Summary answer: Data have shown a differential miRNA activation depending on the IVF stimulation protocol used.
What is known already: Recently, has emerged that miRNAs play a pivotal role in regulating oocyte maturation efficiency during ovarian follicle development. miRNAs modulate several transcripts having important roles in regulating known molecular pathways that determine the fate of ovarian follicle development. It has been reported that miRNAs expression depends on different stimulation protocols. In this view, miRNAs have gained a great scientific interest for the implication in clinical research: they could represent non-invasive biomarkers, prognostic factors and also therapeutic targets.
Study design, size, duration: Ten healthy women with regular cycles and tubal disease or unexplained infertility were included in this study. MII oocytes were retrieved from five patients treated with r-hFSH + r-hLH, and five treated with r-hFSH. Oocyte miRNome variations were evaluated by array cards containing 768 mature miRNA probes.
Participants/materials, setting, methods: Total RNA was extracted from oocytes using mirVana™ miRNA Isolation Kit.

Each experiment was carried out on miRNA pools obtained by mixing the miRNAs from oocyte of five patients treated with rLH + rFSH and five patients treated with r-hFSH alone.

Real time PCR was employed to analyze the miRNA expression level using TaqMan Array Human MicroRNA A + B cards.
Main results and the role of chance: The study of miRNA expression profile of oocytes from women treated with different stimulation protocols provided evidence that r-hLH + r-hFSH or r-hFSH alone produce different effects in terms of regulation of gene expression.

r-hLH + r-hFSH modulates miRNAs (miR-130a, miR-21, miR-15, miR-10a, and Let-7 family members) involved in oocyte reprogramming, proliferation of granulosa cells and the bidirectional cross-talk with cumulus cells.

r-hFSH regulates miRNAs (miR-135a, miR-98, miR320, miR-106a, and Let-7b) involved in the regulation of oocyte maturation, embryonic development and granulose cells steroidogenesis. Ingenuity pathway analysis showed that both treatments are able to modulate miRNAs involved in P53 pathway.
Limitations, reasons for caution: This research included few samples and could be considered as a pilot study.
Wider implications of the findings: Our results suggest that the two drugs affects the expression of different miRNAs playing a crucial role in periovulatory events and could influence the developmental competence of the oocyte and granulose development.
Trial registration number: NA

P-456 *In vitro* fertilization treatment outcomes following standard 2D ultrasound vs. 3D automated follicle tracking (sonoAVC), a prospective randomized study

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Study question: To compare three-dimensional ultrasound imaging of follicular volume to the standard two-dimensional ultrasound follicular surveillance during *in vitro* fertilization (IVF) treatment in a randomized control study.
Summary answer: Implementation of 3D sonographic measurements during follicular surveillance in *in vitro* fertilization treatment was not found to increase oocyte maturation, fertilization rate or pregnancy rate.
What is known already: Use of Sono AVC leads to a significantly lower intra- and inter-observer variability with a significant advantage in time gain for both the doctor and patient. It offers the possibility of continuous training and standardization of follicular monitoring. Sono AVC was found to be highly reliable for ovarian reserve testing by measurement of antral follicle count. Manual and automated follicular measurements during IVF treatment are reported to be comparable, however not in a randomized control trial.
Study design, size, duration: A prospective randomized control study. 54 women undergoing IVF treatment were randomly assigned to undergo follicular measurements with either 2D or 3D ultrasound imaging. Demographic, treatment and laboratory characteristics were collected.
Participants/materials, setting, methods: All patients underwent a fixed antagonist protocol and sonographic assessments were performed throughout ovarian stimulation up until day of oocyte retrieval. Treatment parameters including number of ovarian follicles, diameter/volume averages and endometrial thickness were assessed.
Main results and the role of chance: Mean age, cause of infertility, cycle parameters including duration of treatment, total dose of gonadotropins, large diameter follicles (>13 mm), peak estradiol levels were similar in both groups. The number of sonographic measurements were also similar in the 2D and 3D groups (3.2 ± 1 and 3.6 ± 1.2, $p = 0.1$, respectively). The number of retrieved oocytes (9.9 ± 5.2 and 10.4 ± 5.5, $p = 0.7$), number of mature oocytes (7.7 ± 4.3 and 8.3 ± 4.5, $p = 0.62$), ICSI fertilization rate (0.7 ± 0.3 and 0.79 ± 0.22) and pregnancy rates (17/27–63% and 12/27–44%, respectively, $p = 0.172$) were comparable between the two groups. In a multivariate logistic regression analysis, sonographic tracking method (2D or 3D) was not a significant predictor for follicular maturation rate or achieving a pregnancy.
Limitations, reasons for caution: While the number of participants is small, this is a prospective randomized control study evaluating the efficacy of 3D sonographic follicular surveillance in *in vitro* fertilization.
Wider implications of the findings: 3D sonographic follicular volume measurement was not found to be superior to standard 2D follicular measurement during in-vitro fertility treatment for increasing follicular maturation rates or pregnancy rates.
Trial registration number: ClinicalTrials.gov Identifier: NCT01723267.

P-457 Cohesins SA1/SA2 expression profiling and DNA telomere sizing of cumulus cells as potential indicator of oocyte quality and embryo competence.

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Study question: Are the relative telomere length and cohesins SA1/SA2 mRNA quantitative evaluation of cumulus cells predictive biomarkers of oocyte quality and embryo development potential?
Summary answer: The relative telomere length and expression profiling of cohesins SA1/SA2 of cumulus cells are eligible biomarkers of oocyte competence in ART scenario.
What is known already: Cohesin-SA1 and SA2 form a protein complex that mediates sister chromatid cohesion at telomere termini (SA1) and alongside chromatid arm (SA2), allows chromatin accessibility to regulate gene transcription and triggers DNA repair in G2. Inadequate expression of cohesion SA1 by ageing or cytoplasmic immaturity may led to telomere shortening (defective replication) and affect oocyte quality and embryo competence.
Study design, size, duration: Collectively 280 cumulus/oocyte complex samples were recovered from a total of 51 patients (<38 years old) undergoing different stimulations protocols (FSH, FSH + LH, recombinant or extractive). According to the number of retrieved follicles/patient from 3 to 8 cumulus cells were pooled for the analysis.

Participants/materials, setting, methods: DNA and total mRNA were extracted from cumulus cells and assayed for telomere sizing and SA1/SA2 expression profiling by qPCR and qRT-PCR. The quantification of telomere DNA was accomplished to a single copy housekeeping gene to generate a telomere/single copy gene ratio. Expression levels of SA1/SA2 cohesins and telomere length measurements of samples were ranked in relation to the clinical setting parameters (BMI, age, hormonal protocol stimulation) and oocyte development indicators (embryo quality, reproductive outcome).
Main results and the role of chance: Cohesins SA1 and SA2 mRNAs were both expressed in all cumulus cells analyzed in this study, even if their expression level resulted significantly different. On the whole, SA2 expression was significantly increased in comparison to SA1; moreover, SA2 mRNA resulted more expressed in young (<35 years old) in comparison with old patients (>38 years old). When the expression level was evaluated according to embryo quality and positive reproductive outcome, we observed an increase of the SA2 levels in cumulus cells. We also analyzed the relation between both cohesins mRNA levels and telomere length in the same samples, highlighting a significant raise of SA1 expression level in cumulus cells were the telomere sequence were increased. The same analysis did not revealed any significant correlation with SA2 mRNA expression level.
Limitations, reasons for caution: Only a limited number of single cumulus/oocyte complex were analyzed. Results from pooled cumulus, as gathered in the majority of cases of the present study, may not be fully predictive of single oocytes ontogeny.
Wider implications of the findings: To our knowledge this is the first study addressing the issue of cohesins SA1 and SA2 expression in cumulus cells together with the telomere relative length sizing as biomarkers of oocyte competence. The reported observations may stimulate further research to uncover the role of cohesins in oocytes aneuploidies.
Trial registration number: Not applicable.

P-458 Transplantation of ovarian tissue in high risk patients treated for leukemia; safe approach resulting in ovulations, IVF cycles and pregnancy

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Study question: Patients with leukemia are at high risk for transfer of malignant cells with the transplanted ovarian tissue.

Can we provide safe and effective OTCP-transplantation in patients with leukemia?
Summary answer: However, these young patients are treated with high dose chemotherapy and would highly benefit from the ovarian tissue cryopreservation (OTCP) technique.
What is known already: OTCP-Orthotopic transplantation is an effective technique to restore fertility. Young patients with leukemia are frequently treated with high dose chemotherapy would highly benefit from OTCP. However as studies indicate risk of leukemic cells contamination in the graft, use of this technology has been avoided. Chemotherapy pre-OTCP reduces the risk of leukemic cells presence in the graft. Histology, immunostaining, molecular markers and animal studies can be used to reduce risk of possible infiltration by leukemic cells.
Study design, size, duration: During twelve year period a cohort of 490 patients underwent OTCP in a tertiary oncology reproductive center. Forty six patients had the diagnosis of leukemia and thirteen of these patients were interested in transplantation of stored tissue in order to conceive.
Participants/materials, setting, methods: Eleven patients had acute myeloid leukemia (AML), 2 had chronic myeloid leukemia (CML). Seven AML patients had known molecular markers, bcr-abl translocation was evaluated in CML. Molecular studies used RT-PCR, next generation sequencing (NGS) and novel Trusight Myeloid Sequencing Panel (Illumina). SCID mice transplantation was performed only in AML.
Main results and the role of chance: Histology indicated ovarian follicles despite pre-harvesting chemotherapy exposure but no leukemic cells were found. In 7 AML patients with known genetic markers, 1 patient showed positive marker (translocation 15/17) while all others were negative. In 2 AML patients NGS and myeloid panel revealed new markers in original leukemic cells, but ovarian tissue was negative. RT-PCR for bcr-abl was positive in 1 CML patient. Transplantation into SCID mice was negative in all AML patients evaluated.

Ovarian tissue was transplanted in 2 patients (CML and AML). AML patient conceived following IVF and is in her 2nd trimester of normal pregnancy. There is no evidence of cancer recurrence with more than 5 years follow-up of the CML patient and 1 year for the AML patient.
Limitations, reasons for caution: To use new advanced molecular technologies efficiently we must have access to original leukemic cells in order to search for patient specific new markers. NGS technologies and myeloid panel cannot reveal pathognomonic markers in all cases. The cost of searching for cancer cells in the ovary is still high.
Wider implications of the findings: Transplantation technique is an effective technique and can be used cautiously in patients treated for leukemia. The outcomes of OTCP post chemotherapy are not inferior to those obtained in chemotherapy naive patients. Searching for cancer cells in ovarian tissue should be performed in centers specialized in new technologies.
Trial registration number: 7222-09-SMC.

P-459 Reproductive outcome after oocyte banking

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Study question: What are the reproductive outcomes of women after oocyte banking?
Summary answer: The majority of women banking oocytes conceives naturally.
What is known already: Women at risk for premature ovarian insufficiency may increase their chances of future childbearing by banking their oocytes. Published data regarding reproductive follow-up of these women is scarce.
Study design, size, duration: We carried out a cohort study in 2015 in all 292 women who banked oocytes between 2006 and 2013 in our center.
Participants/materials, setting, methods: We retrieved medical data, e.g., indications and outcomes of ovarian stimulation from medical files. We asked all women who banked oocytes during the study period to participate and fill out an additional questionnaire on demographics, diagnosis, gonadotoxic treatment or ovarian surgery received, menstrual cycle and contraception, relationship status, pregnancy attempts and intended use of banked oocytes.
Main results and the role of chance: A total of 202/292 (69%) women responded and 184/292 (63%) women consented to participate and returned the questionnaire. Of these women, 106/292 (36%) were trying or had tried to become pregnant after oocyte banking. In our study 59/106 (56%) women became pregnant. These 59 women reported 75 pregnancies, 45/75 (60%) of the pregnancies were spontaneously conceived and 30/75 (40%) pregnancies were conceived with ART. The 75 pregnancies resulted in 42 (56%) live births, 16 (21%) ongoing pregnancies, 16 (21%) miscarriages and one (1%) induced abortion. From the 30 pregnancies conceived with ART in 8/30 (27%) pregnancies the women used their banked oocytes.
Limitations, reasons for caution: Since this was a single centre study our results might not be generalizable to other settings and populations. An even longer follow-up of the fate of banked oocytes is needed to further explore the efficacy of the treatment.
Wider implications of the findings: Our results suggest that most women wish to become pregnant in the next few years after cryopreservation of oocytes. Considering that most women in our study conceived naturally, the chances of natural conception should be discussed during the counseling of oocyte banking.
Trial registration number: W15_041.

P-460 Chronic inflammation *in utero* may reduce ovarian reserve

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Study question: Does maternal inflammation during pregnancy leads to reduction of the ovarian reserve of the offspring?

Summary answer: Chronic maternal inflammation induced IUGR offspring and a reduction of the offspring' ovarian reserve.
What is known already: There is an association between intrauterine malnutrition and hormonal imbalance and negative consequences on the development of the reproductive system in females. Excess maternal inflammation and oxidative stress while *in utero* have been known to affect gross fetal development. However, an association between the inflammatory process *in utero* and the effects on ovarian development and future fertility has not yet been demonstrated. This study focused on LPS-induced inflammation in early pregnancy and its effect on ovarian development and reserves of the offspring, using a rat model.
Study design, size, duration: An animal model with Sprague-Dawley pregnant rats ($n = 11$) received (50 µg/kg bodyweight) intraperitoneal lipopolysaccharide (LPS group) or saline solution (Control group) on day 14, 16, and18 of gestation. Pups were delivered spontaneously. At 3 months, female offspring were weighed and sacrificed.
Participants/materials, setting, methods: Ovaries were harvested for: 1. Follicle count using Hematoxylin and Eosin staining, 2. Apoptosis: ovaries were stained for Caspase and 3. Serum CRP and AMH levels were determined.
Main results and the role of chance: Birth weight of pups was significantly lower in the LPS group compared to the control group (6.0 mg ± 0.6 vs. 6.6 mg ± 0.4; $P = 0.0003$). The LPS group had lower proportion of preantral follicles, as well as increased intensity of Caspase 3 staining (510 u vs. 155.5 u; $P = 0.007$). There was no significant difference in the CRP levels between the two groups (1.06 vs. 0.96; $P = 0.42$). AMH levels were significantly lower in the LPS group (4.155 ng/ml ± 0.4669 vs. 6.082 ng/ml ± 1.884; $P = 0.016$).
Limitations, reasons for caution: We were not able to count the total number of follicles in the ovaries, we calculated only the proportion of the different follicles in each ovarian section.
Wider implications of the findings: LPS induced inflammation *in utero* maybe linked to a premature depletion in follicular count through a process of premature induction of apoptosis. It highlight the importance of prevention or early treatment of maternal infection/inflammation, because it can lead to a more promising outcome for the future fertility of the offspring.
Trial registration number: Not relevant.

P-461 A predictive nomogram based on multiple biomarkers leads to appropriate rFSH starting dose in IVF/ICSI cycles: a randomized controlled trial

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Study question: What is the role of a nomogram based on multiple biomarkers (age, basal FSH and AMH) to determine the most appropriate rFSH starting dose in IVF/ICSI cycles?
Summary answer: The nomogram allows the choice of an appropriate rFSH starting dose for optimizing the ovarian response.
What is known already: The number of oocytes retrieved is a key prognostic marker in women undergoing IVF/ICSI. Choosing different doses of gonadotropins for different patients is therefore a crucial clinical decision in the treatment of infertile couples. Although tailored therapy based on markers of ovarian reserve is an agreed-upon approach by most, studies suggesting how to determine individualized therapy compared to "one size fits all" are scarce. Recently a nomogram based on female age, FSH and AMH has been proposed to suggest the starting dose (La Marca et al., 2012). In the present study, the nomogram has been externally validated in a RCT.
Study design, size, duration: This RCT involved 194 couples attending their first IVF/ICSI cycle between 2013 and 2015. The patients underwent a long protocol and were randomized to receive rFSH starting dose selected on the basis of their age (150 IU if ≤35 years, 225 IU if >35 years) (control group) or on the basis of the nomogram (study group).

The primary outcome measure was the optimal number of retrieved oocytes defined as 8–14.

Participants/materials, setting, methods: The study was conducted at ANDROS Day Surgery Clinic, Palermo, Italy. Inclusion criteria were: female age 18–40 years, BMI 18–25 kg/m^2, AMH 1.0–4.0 ng/ml, FSH ≤15 mIU/ml. Exclusion criteria were PCOS, irregular cycles, endometriosis, previous ovarian surgery, ovarian cysts, any known endocrinological disease.

It was calculated that a total sample size of 354 achieves 90% power to detect a difference in optimal response of 15% (α-level = 0.05). An interim analysis was pre-planned at 50% of recruitment.
Main results and the role of chance: Data of 194 patients were available for the interim analysis: 99 (control group) and 95 (study group). 191 couples started the cycle (3 couples dropped out in study group).

There were not significant differences between the two groups in age, BMI, infertility years, AFC, AMH, FSH. In the control group, 57 and 43% of patients received the dose of 150 and 225 IU, respectively. In the study group, 48% of patients received 225 IU of rFSH, while 52% received a dose ranging between 125 and 212.5 IU.

The optimal response was observed in 58/92 patients (63.0%) in the nomogram group and in 42/99 (42.4%) in the control group ($p = 0.004$), a difference sufficient for terminating the trial.

Patients with <8 oocytes retrieved were 24/92 (26.1%) in the nomogram group and 40/99 (40.4%) in the control group ($p = 0.04$). Patients with >14 oocytes retrieved were similar between the two groups.

Embryological data and clinical pregnancy rates (CPRs) were similar between the groups.

The CPRs in the whole population were significantly higher in those patients with an optimal response than in those with a non-optimal response both per started cycle (46/100, 46% vs. 15/91, 16.5%, $p = 0.001$) and per embryo transfer (46/95, 48.4% vs 15/56, 26.8% $p < 0.01$).
Limitations, reasons for caution: Since the nomogram is based on hormonal markers that may have some inter-lab degree of variability, a well-designed multicentric trial is needed before the clinical diffusion of the algorithm.
Wider implications of the findings: The nomogram could become the basis for the selection of the most appropriate rFSH dose to obtain an adequate number of oocytes.

Reference
La Marca A. et al. (2012). *BJOG*. 119: 1171–1179.

Trial registration number: ClinicalTrials.gov (ID registration code: NCT01816789).

P-462 Should intrauterine insemination be performed before or after ovulation? – A retrospective analysis of 6701 cycles

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Study question: Is there a difference in clinical pregnancy and live birth rates between inseminations performed before and after ovulation
Summary answer: No differences were found in pregnancy and live birth rates when comparing inseminations performed before and after ovulation.
What is known already: Heterologous Insemination (IUI-H) is an established effective non-invasive treatment for infertile patients. IUI-H is often offered as a first-line treatment of infertility. In 2014, a total of 9498 IUI-H cycles were conducted in Denmark. The correct timing of the insemination around the time of ovulation is important since the human gametes have a limited survival time
Study design, size, duration: A retrospective study using data from 6701 IUI-H treatment cycles from April 1990 to August 2011, performed at the Fertility Clinic, Odense University Hospital, Denmark.
Participants/materials, setting, methods: IUI cycles were either natural or stimulated with Clomifen, FSH or HMG. Ovulation was induced with 6500 IE or 10,000 IE hCG. Vaginal ultrasound at the time of insemination, 38 h after hCG, revealed whether ovulation had occurred. Ovulation was defined as a collapsed or blood filled follicle. The primary outcome of the study was live birth rate, secondary outcome was pregnancy rate.

Main results and the role of chance: In 2128 cycles (31.8%) ovulation had occurred at the time of insemination, whereas in 3870 cycles (57.8%) ovulation had not occurred. In 703 cycles (10.5%) ovulation had occurred only in one ovary. The overall pregnancy rate was 15.5% and the overall live birth rate 12.1%. In cycles where ovulation was absent before IUI, the clinical pregnancy rate was 15.7% and the live birth rate was 12.6%. When ovulation was detected the pregnancy rate was 15.4% and the live birth rate 12.9%. With one-sided ovulation only, the pregnancy rate was 15.5% and live birth rate 10.3%. No significant differences were seen in live birth rates between groups.
Limitations, reasons for caution: The results are based on retrospective observational data, however, from a single fertility center and including a large sample size. Selection bias may be present, and generalizability can be limited. Although the data is older, the same insemination procedures were used all through the period.
Wider implications of the findings: This is the largest study until now, showing that in IUI-H, pregnancy and live birth rates are similar, whether or not ovulation has occurred at the time of insemination. Thus, timing of insemination before or after ovulation as well as ultrasound examination prior to insemination is redundant.
Trial registration number: Not relevant.

P-463 Pioglitazone is effective for multiple phenotypes of the Zucker fa/fa rats used as a model of polycystic ovary syndrome with insulin resistance

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Study question: What effects, other than on insulin sensitivity, does treatment with pioglitazone, a thiazolidine derivative, have on a rat model of PCOS induced by insulin resistance?
Summary answer: Pioglitazone treatment reduced the percentage of atretic follicles and serum anti-mullerian Mullerian hormone levels, and stimulated the serum adiponectin level in Zucker fa/fa rats.
What is known already: Hyperandrogenism and insulin resistance may be related to the etiology of PCOS. Zucker fa/fa rats complicated with polycystic ovary, are obese, have insulin resistance without diabetes mellitus or hyperandrogenism and can be utilized as a PCOS rat model without effects of hyperandrogenism. PCOS patients are reported to have elevated serum AMH and low serum adiponectin levels. Excessive AMH is reported to interfere with follicle growth in these patients and adiponectin blocks apoptosis in some tissues. Pioglitazone is administered to PCOS patients with insulin resistance to induce ovulation but the mechanisms by which it does have not been elucidated.
Study design, size, duration: We purchased female Zucker fa/fa rats, 4 weeks age, and also as well as Zucker$^{+/+}$ rats for use as normal control rats with normal insulin sensitivity, and then administered pioglitazone or vehicle to groups of 5 rats of each type (4 groups in all). After 2 week of treatment, they were sacrificed for the study.
Participants/materials, setting, methods: From the dead rats, we got obtained serum samples and both ovaries. We checked determined the body weight, ovarian weight, and examined the serum AMH, adiponectin, testosterone, androstenedione levels. And we also examined ovarian histology to check follicle numbers by using HE staining, and check the number of atretic follicles using TUNEL methods.
Main results and the role of chance: Zucker fa/fa rats are significantly heavier than of Zucker$^{+/+}$ rats ($p < 0.05$), but pioglitazone treatment did not influence body weight in either group, nor did it influence ovarian weight. However, the total number of follicles was significantly increased in Zucker fa/fa rats compared with Zucker$^{+/+}$ rats ($p < 0.05$). Pioglitazone treatment did not alter the total number of follicles in Zucker fa/fa rats, but did significantly decrease the number of atretic follicles in Zucker fa/fa rats ($p < 0.05$). The serum AMH was significantly higher in Zucker fa/fa rats than in Zucker$^{+/+}$ rats ($p < 0.05$). Pioglitazone treatment significantly decreased the serum AMH level and significantly increased the serum adiponectin level in Zucker fa/fa rats ($p < 0.05$). Serum testosterone and androstenedione levels are quite low in Zucker fa/fa rats, and were not influenced by pioglitazone treatment.
Limitations, reasons for caution: In this study we examined the effects of pioglitazone treatment in a rat model of PCOS but not in humans.
Wider implications of the findings: In this study, pioglitazone reduced the serum AMH level and promoted follicle growth. In addition, it appeared to block follicle atresia via elevation of serum adiponectin. We have thus, for the first time. demonstrated that the effectiveness of pioglitazone treatment for PCOS occurs via the mechanisms described above.
Trial registration number: Our study is not a clinical trial.

P-464 Resting heart rate changes during the menstrual cycle

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Study question: Can we accurately and precisely detect different phases of cycle through resting heart rates provided by electronic wearable technology?
Summary answer: Electronic wearable technology measuring number of daily steps/distances, sleep and parameters such as heart rate can be used to determine different phases of menstrual cycle.
What is known already: It is known that due to different reproductive hormone concentrations and dominances (determined as ratios between 2 hormones) during the menstrual cycle there is a vast physiological response, such as higher water retention (and consequently changes in weight, electrolytes, pH and perspiration), as well as changes in metabolism, ligament flexibility, vision and other.

The observation that resting heart rate (RHR) changes through different phases of menstrual cycle, reported by Moran and colleagues was a basis for this project.

According to their research RHR was significantly higher in both ovulatory and luteal phase when compared to menstrual and follicular phase.
Study design, size, duration: We have collected data on resting heart rate, ovulation, as well as menstrual cycle length and period duration. We have collected up to ten menstrual cycles of individual data on five cycling individuals, as well as three negative controls (either females on hormonal birth control or a male participant) aging 25–40 years.
Participants/materials, setting, methods: We used two mobile phone applications to collect data: Clue and FitBit

Resting heart rate (RHR) measurement was obtained using electronic wearable bracelet: *FitBitHR* (we allowed 7–10 days of "getting to used" time when first started to use).

Ovulation measurement-using Clearblue dual hormone indicator ovulation tests (detecting estradiol rise and luteinizing hormone surge).
For period and other entries-Clue application.

Exclusion: if any of the ailment categories was entered in Clue app on a given day.
Main results and the role of chance: We have observed similar resting heart rate (RHR) patterns as described by Moran and colleagues:

If we divide menstrual cycle in 4 phases: bleeding phase, follicular phase, ovulation and luteal phase.

Starting with the bleeding phase (the period) resting heart rate lowers towards the follicular phase, when it is the lowest throughout the entire menstrual cycle.

Resting heart rate starts to increase a few days before ovulation, and continues rising throughout the luteal phase.
At the start of the next cycle, resting heart rate starts dropping.

In the negative controls resting heart rate was not showing such patterns, but was more dependent on the overall health and activity of a given participant.
Limitations, reasons for caution: We observed that ailments such as cold, or fever have a strong impact on resting heart rate, but not ovulation and such results should be carefully examined or in the cases where the ailment lasted for more than 2 days discarded.
Wider implications of the findings: Findings presented here are the pilot and a start of a large-scale study that would take in consideration different age groups and lifestyles and try to determine if this phenomenon is found independently of the lifestyle differences.
Trial registration number: –

P-465 The oocyte utilization rate (OUR) a measure of pregnancy potential: multi-centre analysis of 7703 cycles

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Study question: How many mature (MII) oocytes obtained after ICSI are required to achieve an ongoing pregnancy in different age classes of women undergoing ICSI?
Summary answer: The oocyte utilization rate (number of clinical pregnancies per mature oocyte) remains stable in women upto age 37. Beyond 38 years, OUR significantly decreased.
What is known already: The clinical pregnancy and live birth per mature MII oocyte have been proposed to be valuable criteria to evaluate individual ART efficiency and to improve patient management. However, the number of human oocytes obtained from IVF/ICSI treatments that are competent to develop to a clinical pregnancy and live birth has been reported to be low, in the region of 2–5% depending on patient age. There is however no data at a national level on this measure and therefore to provide a starting point for subsequent initiatives to improve treatment outcomes the current multi-centre analysis was carried out.
Study design, size, duration: This was a multicentre retrospective audit on anonymised outcome data captured in an Exel file, then merged and analysed centrally. Overall data came from 6814 females who had 7703 oocyte retrievals. Data was captured from ICSI only treatment cycles in which ovarian stimulation was with follitropin alfa. Data capture was from 2011 to September 2015. Additional outcome information was obtained from subsequent vitrified embryo transfer cycles carried out in the same female cohort.
Participants/materials, setting, methods: Five French IVF centres having been involved in Fertility treatment for over 10 years participated. Female age was categorized into 5 groups (25–29 years; 30–34; 35–37; 38–39 and ≥40). Main assessment criterion was clinical pregnancy. Nonparametric LOESS method was used to estimate the relationship between pregnancy and the number of MII oocytes (on all attempts). Relationship between pregnancy at 1st attempt and female age and number of MII oocytes was estimated by logistic regression.
Main results and the role of chance: The mean female age was 33 ± 5 years and 48% of cycles were the first attempt and only a few (5.7%) were in the rank of 5 or more. There were in total 1892 clinical pregnancies and 341 from subsequent vitrified-thawed cycles. The pregnancy rate increased with the number of MII oocytes (up to 20), but in women older than 38 with high numbers of MII oocytes retrieved, it is not possible to give an accurate estimate in these age classes. Taking into account all fresh and frozen pregnancies, the number of MII oocytes required to achieve a pregnancy remained relatively stable below 37 years (25–29, 21.7; 30–34, 21; 35–37, 19.8) and then increased dramatically in subsequent age classes: 28.7 at 38–39 years and 30.8 at 40 years or more. These numbers revealed that the pregnancy cumulative OUR tended to increase from 4.6% (years 25–29) to 5% (35–37) and then decreased to 3.2% in women 40 or older. In a multiple logistic regression analysis on the first attempt, female age ($p < 0.0001$) and number of retrieved MII oocytes ($p < 0.0001$) were negatively and positively related to pregnancy, respectively.
Limitations, reasons for caution: Each cycle was considered as independent. Furthermore, over 30% of the cryopreserved embryos have not been used yet, representing residual clinical pregnancy and live birth potential not evaluated by the data.
Wider implications of the findings: Our results should lead to improve couple management. Moreover, these data may provide a tool to estimate the number of metaphase II oocytes needed to achieve a clinical pregnancy and live birth in case of female fertility preservation
Trial registration number: This was a retrospective clinical audit.

P-466 Anti-Müllerian hormone levels and female fecundity – results from a prospective cohort study comprising 654 women with a follow-up period of four years

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Study question: Can anti-Müllerian hormone (AMH) levels predict fecundity in women?
Summary answer: Serum-AMH was not an age-independent predictor of female fecundity.
What is known already: Women seek assessment regarding their expected reproductive lifespan. AMH is a well-established predictor of ovarian response and, to some extent, to pregnancy rates after assisted reproductive technologies (ART). AMH is associated with age at menopause, but it remains uncertain whether AMH is an independent predictor of fecundity in women from the background population.
Study design, size, duration: A prospective cohort study comprising 863 women aged 20–41 years recruited from 2008 to 2010 of whom 709 (79%) participated in a questionnaire-based follow-up study four years after inclusion. The follow-up questionnaire was emailed to participants consecutively from September 2012 through February 2014, 4 years after the initial examination. The mean ± SD follow-up period was 4.0 ± 0.2 year. Women who were pregnant at the time of inclusion ($n = 55$) were excluded, leaving 654 women for analysis.
Participants/materials, setting, methods: At inclusion, the ovarian reserve was assessed by AMH and antral follicle count on Cycle Day 2–5, and baseline data were collected on reproductive and medical history, demography, and lifestyle. After four years, data were collected on subsequent conceptions, time to pregnancy (TTP), births, unsuccessful attempts to conceive, and fertility treatments. AMH was categorized in 1st (low), 2nd–9th (normal), and 10th (high) tentiles. Fecundability ratios (FR, 95% confidence intervals (CI)) were calculated by discrete-time survival-analysis.
Main results and the role of chance: At inclusion, the 654 women had a mean ± SD age of 32.7 ± 4.4 years; 363 (56%) had conceived and 306 (47%) had a history of ≥1 livebirths. The median (90% population limits) AMH-levels (pmol/l) were: 5.0 (1.1; 6.5); 19.9 (7.7; 43.2); and 65.2 (51.0; 99.7) in the low, normal and high AMH-group. During the 4-year follow-up period, 242/654 (37%) had not attempted to conceive, 359/654 (55%) had conceived at least once, 63/359 (18%) by means of fertility treatment, and 46/654 (7%) reported an unsuccessful attempt to conceive with a median (90% population limits) length of 16 (1; 72) months. Of the 412 women, who had conceived or attempted to do so, 80 (19%) reported a TTP or time of trying that exceeded 12 months. Low AMH was associated with reduced odds for conception (OR 0.5, 95% CI: 0.3; 0.8), but there was no association between AMH and conception after age-adjustment (aOR 0.7, 95% CI: 0.4; 1.3). Furthermore, TTP and the time of trying was prolonged in women with low AMH (FR 0.5, 95% CI: 0.3; 0.8), but not in women with high AMH (FR 1.3, 95% CI: 0.9; 1.9). However, fecundability was independent of both low (FR 0.7, 95% CI: 0.4; 1.1) and high (FR 1.1, 95% CI: 0.8; 1.6) AMH-levels in the age-adjusted model.
Limitations, reasons for caution: Data on TTP and unsuccessful attempts to conceive were collected retrospectively, which implies a risk of recall bias. The participants were health care personal which may limit the extrapolation to other populations. Additionally, the data do not allow analysis beyond the age of 45 years at follow-up.
Wider implications of the findings: Our data suggests that overall AMH adds little predictive value to female age when advising women on their future fecundity. It remains important to further develop reliable methods to identify women at risk of infertility, including those at risk of premature ovarian insufficiency, to prevent unwanted childlessness.
Trial registration number: Not applicable.

P-467 Oncologists' and haematologists' perceptions of fertility-related communication – a nationwide survey

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Study question: What are the main factors influencing the likelihood of physicians discussing fertility-related issues with female and male cancer patients of reproductive age?
Summary answer: The majority of oncologists and haematologists address fertility issues but important reasons for not doing so including patient factors and organizational barriers like high workload.

What is known already: Many cancer treatments have a negative impact on fertility. Despite international guidelines recommending health care professionals to bring up fertility-related issues with cancer patients of reproductive age many patients, especially women, report inadequate information provision. Previous research from the physician perspective has been mainly descriptive or examining the influence on single factors on communication behavior.
Study design, size, duration: The present study is a cross-sectional, nationwide survey study targeting all physicians registered as working in cancer care in Sweden (n = 821). In 2015 all eligible physicians were approached regarding anonymous participation in a survey about fertility-related communication with patients of reproductive age (women 18–45 years; men 18–55 years). The questionnaire was available both on paper and via a web link.
Participants/materials, setting, methods: Physicians registered as working in cancer care were eligible; exclusion criteria were no recent clinical activity and very few patients of reproductive age. Total response rate of 55%. A questionnaire was developed on the basis of two US surveys and adapted to the Swedish context. Questions assessed practice behaviour, perceived barriers and attitudes towards fertility communication, and confidence in knowledge regarding treatment-related fertility risks. Data were analysed with Chi-Square tests and logistic regression.
Main results and the role of chance: More than 90% of responding physicians agreed that discussing fertility is their responsibility and a majority stated often or always discussing fertility-related issues with patients of reproductive age. Multivariate regression models were performed to investigate factors associated with not routinely discussing treatment-related fertility risks with patients of reproductive age, which was reported by 27% of surveyed physicians for female patients and 30% of physicians for male patients. The factors significantly associated with not routinely discussing fertility risks were: the patient already having children, a high workload, seeing 5 patients of reproductive age weekly, and having access to a reproduction clinic at the hospital (both sexes). Additional factors associated with a lower frequency of fertility discussions were: the need to start cancer treatment immediately, and the physician having <5 years experience from cancer care (female patients only), as well as a low confidence in knowledge regarding treatment-related fertility risks (male patients only). In addition, a patient presenting with a poor prognosis was by 90% of physicians perceived as a circumstance that would make it unlikely for them to bring up fertility issues.
Limitations, reasons for caution: While the relatively low response rate (55%) is comparable to that of other studies targeting physicians, it limits the potential for generalizing results to the entire population. As the survey was anonymous in order to avoid social desirability responding, comparison of responders and non-responders was not possible.
Wider implications of the findings: The present results suggest that clinical circumstances constitute important reasons for not discussing fertility issues with cancer patients of reproductive age. However, some of the factors related to a low frequency of fertility discussions should be possible to alter through organizational changes or educational efforts.
Trial registration number: N/A.

P-468 Cumulative live birth rates (CLBR) after first ART cycle including subsequent frozen-thaw cycles in 1050 women attending a randomized trial comparing GnRH-antagonist versus GnRH-agonist protocol

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Study question: Is CLBR similar in GnRH-antagonist and GnRH-agonist protocol in first ART cycle including subsequent frozen-thaw cycles from same oocyte retrieval?
Summary answer: Chances of at least one live birth following utilization of fresh and frozen embryos after first ART cycle are similar in GnRH-antagonist and GnRH-agonist protocol.
What is known already: Reproductive outcomes of ART treatment are traditionally reported as pregnancies per cycle. However, the primary concern is the overall chance of live birth. After first fresh ART cycle our results showed LBR of 22.8% and 23.8% (P = 0.70) for GnRH-antagonist and GnRH-agonist, respectively. But with CLBR including both fresh and frozen embryos from first oocyte retrieval, chances of at least one live birth increases. There is no previous Randomized controlled trial (RCT) comparing CLBR in GnRH-antagonist versus GnRH-agonist protocol. Previous studies on CLBR are either retrospective cohort studies including multiple fresh cycles or RCT comparing SET with DET showing CLBR around 50%.
Study design, size, duration: Phase IV, dual centre, open-label, RCT including 1050 women allocated to short GnRH-antagonist or long GnRH-agonist protocol in a 1:1 ratio over a five-year period with a minimum of 2-years followup. The aim was to compare CLBR between the two groups following utilization of all fresh and frozen embryos from the first ART cycle.
Participants/materials, setting, methods: Women referred for first ART cycle <40 years of age were included. All started standardized ART protocols with fixed rFSH dose according to age, planned day-2 SET and freezing of additional embryos. Frozen embryos were used in subsequent frozen-thawed cycles. CLBR is defined as at least one live birth per allocated woman after fresh and frozen cycles. Subjects were censored out after first live birth. Sub-group analyses were performed for stratified BMI and age categories.
Main results and the role of chance: When combining all fresh and frozen-thaw embryo transfers from first oocyte retrieval with a minimum of 2-years followup, the CLBR to first delivery was 33.3% (178/534) in the GnRH-antagonist group versus 30.6% (158/516) in the GnRH-agonist group (P = 0.34; OR:1.13; 95% CI:0.87 to 1.47).

Mean time to first live birth was 10.6 months in the GnRH-antagonist group compared to 11.3 months in the GnRH-agonist group (P < 0.01). Number of women with a secondary live birth was n = 7 and n = 12 for GnRH-antagonist and GnRH-agonist, respectively. At the end of trial n = 11 had unused frozen embryos and still no live birth in the GnRH-antagonist group compared to n = 9 in the GnRH-agonist group.

The stratified BMI groups were lean BMI <25 kg/m^2, overweight BMI 25–30 kg/m^2 and obese BMI>30 kg/m^2. Similar CLBR were found for lean women (36.9 vs. 33.8%) and women with overweight (30.1 vs. 37.7%), but for obese women CLBR was significantly higher in the GnRH-antagonist group 32.0% (24/75) vs. 12.0% (10/83) in the GnRH-agonist (P < 0.01).

No differences were found in the stratified age groups =<36 and >36 when comparing GnRH-antagonist with GnRH-agonist.
Limitations, reasons for caution: The duration of the trial is a limitation with introduction of new methods as "Freeze all," "GnRH-agonist triggering," and blastocyst culture of surplus embryos for freezing. However the RCT-design ensured that new methods were introduced to both groups simultaneously. Further, with minimum 2-years follow-up a minority still have cryopreserved embryos.
Wider implications of the findings: With the improvement of embryo culture, freezing and thaw methods as well as a strategy of elective single embryo transfer, CLBR until first live birth provides an all-inclusive success rate for ART. When comparing GnRH-antagonist and GnRH-agonist protocol we find similar CLBR.
Trial registration number: EudraCT #: 2008-005452-24.
ClinicalTrial.gov: NCT00756028.

P-469 Angiotensin-(1–7) in human follicular fluid correlates with oocyte maturation

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Study question: Do angiotensin (Ang)-(1–7) levels in human ovarian follicular fluid (FF) correlate with the number and proportion of mature oocytes obtained for *in vitro* fertilization (IVF)?
Summary answer: Ang-(1–7) levels in human FF correlate not with the total number but with the proportion of mature oocytes collected upon ovarian stimulation for IVF.
What is known already: Angiotensin (Ang)-(1–7) is an active peptide of the renin-angiotensin system that stimulates oocyte maturation in

isolated rabbit and rat ovaries. However, its role in human ovulation remains unexplored.

Study design, size, duration: This was a prospective cohort study including 64 participants from a single IVF center. Sample size was calculated to achieve a statistical power of 90% in detecting meaningful quantitative differences between group medians. The participants were enrolled in the study during six consecutive months. Plasma samples were obtained from all subjects at day 21 of the last menstrual cycle before starting pituitary blockade and controlled ovarian stimulation (COS), and immediately before oocyte pickup.

Participants/materials, setting, methods: Plasma and FF samples were quickly mixed with a protease inhibitor cocktail and stored at –80°C. Ang-(1–7) was quantified in plasma and FF samples by a highly sensitive and specific radioimmunoassay. FF Ang-(1–7) levels were stratified into tertiles and the patients of each tertile were compared for COS/IVF outcomes using Kruskal-Wallis analysis of variance. Multiple regression analysis was used to adjust correlations for potential confounders.

Main results and the role of chance: There was a fourfold increase in plasma Ang-(1–7) after ovulation induction (median 160.9 vs. 41.4 pg/ml, $p < 0.0001$). FF Ang-(1–7) levels were similar to (169.9 pg/ml) but did not correlate with plasma Ang-(1–7) levels ($r = –0.05$, $p = 0.665$). Patients at the highest FF Ang-(1–7) tertile had a higher proportion of mature oocytes compared to patients at the lower FF Ang-(1–7) tertile (median 100% vs. 70%, $p < 0.01$). There was a linear correlation between FF Ang-(1–7) and the proportion of mature oocytes ($r = 0.380$, $p < 0.01$), which remained significant after adjustment for age and duration of infertility ($r = 0.447$, $p < 0.001$).

Limitations, reasons for caution: This is an observational study, therefore no causal relationship can still be established between Ang-(1–7) and human oocyte maturation.

Wider implications of the findings: The present study shows for the first time that Ang-(1–7) levels in human FF correlate with the proportion of mature oocytes after ovarian stimulation for IVF. Since this peptide promotes oocyte maturation in other species, it deserves further investigation as a potential maturation factor to human oocytes.

Trial registration number: Does not apply.

P-470 An approach to calculate the number of MII oocytes required to guaranty IVF success in a fertility preservation programme

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Study question: How many MII oocytes are needed to reach a reasonable likelihood of pregnancy in non-infertile women?

Summary answer: In good prognosis patients 7,7 MII oocytes were needed per implanted embryo and 9,1 per baby born.

What is known already: Oocyte vitrification for fertility cryopreservation is now frequent in IVF clinics. Patients need to be counseled about the probabilities of success as a function of the number of MII oocytes stored. The direct ratio of implanted gestational sacs per MII oocyte delivers a pessimistic message because infertile patient population is biased towards lower pregnancy results than in fertility preservation patients.

Study design, size, duration: The ratio of implanted gestational sacs with heart activity per MII oocyte was calculated in patients with at least one ongoing implantation in their IVF cycles. From 2006 to 2014 a total of 1608 own oocyte IVF cycles were included in the study. Only frozen embryo transfer performed before 2016 were analyzed.

Participants/materials, setting, methods: All cycles performed to women who underwent their first IVF treatment aged <40 years and achieved at least one ongoing pregnancy after fresh or frozen embryo transfer. The number of metaphase II oocytes in the 3.7% conventional IVF cycles of this study was calculated according to the MII/Total oocyte ratio in the ICSI cycles. This calculation together with the observed in ICSI cycles gave a total of 12.293 MII oocytes.

Main results and the role of chance: A total of 916 ongoing pregnancies were obtained after fresh embryo transfer and 387 after frozen embryo transfer. Overall the observed gestational sacs with heart activity were 1.588. The ratio of MII oocytes per gestational sac was 7.7. The number of babies born was 1.351 and the ratio of MII oocytes per baby born was 9.1. The remaining frozen embryos in this group of patients are 1.149 (68% of those already thawed), this can lower the ratio of MII oocytes needed for a successful outcome.

Limitations, reasons for caution: This is a retrospective study. The population of infertile patients undergoing IVF treatment is not comparable to fertile women who need to postpone their maternity, however, the patients included in this study, who obtained at least one pregnancy, are more likely to resemble the success likelihood of fertile women.

Wider implications of the findings: The data presented here can be a tool for counselling women who need to cryopreserve their fertility either for medical or social reasons. Another application of these results can be the quality control evaluation of oocyte wastage in relation to the stimulation regime of the clinic.

Trial registration number: NA.

P-471 Impact of thyroid autoimmunity on fertility outcome in IVF patients

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Study question: What is the effect of anti-thyroperoxidase antibodies on live birth rate after *in vitro* fertilisation?

Summary answer: No significant difference was seen in pregnancy-, delivery- or miscarriage rate between the TAI positive and TAI negative group.

What is known already: Currently available data support that it is reasonable to test TSH and anti thyroperoxydase antibodies in infertile women attempting pregnancy. However data regarding the outcome of fertility treatment in the presence of thyroid autoimmunity are conflicting and the optimal upper value of TSH in the presence or the absence of thyroid autoimmunity (TAI) remains debated.

Study design, size, duration: Cohort study. A two armed study design was performed: patients with TAI and patients without TAI. We included all patients who start their first IVF cycle in our center between January 2010 1st and 31 December 2014 with follow-up of outcome until 31 December 2015. Live birth delivery after 25 weeks of gestation is taken as the primary endpoint of our study.

Participants/materials, setting, methods: A total of 3254 patients underwent their first IVF/ICSI cycle between 01/01/2010 and 01/06/2014. Patients with clinical thyroid dysfunction were excluded, as were patients under treatment with levo-thyroxine or antithyroid drugs. Primary endpoint is the effect of TAI on live birth rate. Secondary endpoint is the effect of TSH on live birth rate, according to TAI status of patient.

Main results and the role of chance: After exclusion 3254 patients were finally included in the study. 340 had a TAI positive and 2914 a TAI negative status. There was no significant difference in mean age, BMI nor in AMH. Thyroid function revealed a significantly higher TSH level in the TAI positive group compared to the TAI negative group (2.01 vs. 1.72 mIU/L). Of the 340 patients in the TAI positive group 39% ($n = 134$) became pregnant. Of the 2914 patients in the TAI negative group 36% ($n = 1037$) became pregnant.

Chi squared testing did not show any significant difference ($p>0.01$) between the TAI positive and TAI negative group in respect to pregnancy-, delivery- or miscarriage rate. Moreover there was no significant difference in pregnancy-, delivery- or miscarriage rate when comparing subgroups according to TSH level (TSH ≥ 2.5 mIU/L vs TSH <2.5 mIU/L). Cox regression analysis in the TAI positive group, with live birth delivery as the outcome variable and age, fT4, TSH, BMI, smoking habits, ovarian reserve and type of stimulation as explanatory variables, indicated that thyroid function (TSH) did not influence delivery rate. Only age had a significant and independent effect on delivery rates.

Limitations, reasons for caution: Retrospective design of this study. Absence of region-specific reference ranges for thyroid hormones and the absence of follow-up of TSH values during ART and subsequent pregnancy.

Wider implications of the findings: Actually a lot of patients receive a treatment with thyroid hormones once the TSH is above 2.5 ml/L. We believe that overtreatment in this population can be avoided.

Trial registration number: n/a.

POSTER VIEWING SESSION

MALE AND FEMALE FERTILITY PRESERVATION

P-472 Prospective, multicentric study of AMH decay during chemotherapy for breast cancer treatment in 134 young women of reproductive age

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Study question: The aim of the study was to describe serum AMH dynamics throughout chemotherapy (CT) in young women of reproductive age treated for breast cancer.
Summary answer: Serum AMH rapidly falls in young women treated for breast cancer with (neo)adjuvant chemotherapy.
What is known already: Serum AMH has been shown to be very low in women of reproductive age after CT treatment in various cancer types, including a few ones in breast cancer. While all studies are consistent in reporting such a fall in serum AMH levels after CT in breast cancer, most of them were conducted in women aged 40 or more on average, and very few of them performed a longitudinal follow-up of serum AMH during CT protocol.
Study design, size, duration: This observational, longitudinal, prospective, multicentric, cohort study was conducted between January 2010 and July 2011. Eligible patients were 18 to 39 years of age suffering from breast cancer treated with CT according to the FEC/T regimen (Fluorouracil, Epirubicin and Cyclophosphamide [cycles 1–3], Docetaxel [cycles 4–6]). Serum AMH concentration was measured at each chemotherapy administration using AMH/MIS (Beckman Coulter) according to the manufacturer's instructions. All assays were centralized in one lab.
Participants/materials, setting, methods: Women aged 18–39 years treated by FEC/T regimen in one of the 11 participating French Cancer Institutes (Unicancer network) were included. Women with serum AMH <0.42 µg/L (n = 6) or >7 µg/L (n = 25) on diagnosis were excluded from the subsequent analysis on AMH decay during CT. Prospective data (demographic, clinical, histological and menstrual data) were extracted.
Main results and the role of chance: A total of 92.7% of the patients were diagnosed with ductal carcinoma. On diagnosis, women were aged 35.3 ± 3.7 years on average. Mean serum AMH was 2.84 ± 1.64 ng/mL on diagnosis, and sequentially decreased to 1.63 ng/ml (49.2% relative decrease of initial level), 0.78 ng/ml (84.5% decrease) and 0.58 ng/mL (94%) after each FEC administration, followed by 0.50 and 0.46 ng/mL (96% decrease) after each Docetaxel administration. The proportion of patients with undetectable serum AMH rose from 7.4% after first FEC administration to 79.7% after the last Docetaxel administration. Overall, patients <35 years old presented with a slower initial AMH decrease rate than patients ≥35 years, respectively −39% vs. 55% after first FEC treatment and −78 vs −88% after second FEC treatment. On average, serum AMH became undetectable after 3 CT treatments in women <35 years and after 2 CT treatments in women ≥35 years. AMH decay rate was comparable in women with serum AMH<2 ng/ml on diagnosis and those with 2 < AMH <7 ng/ml. Amenorrhea was observed in 63.6% patients after completion of CT and Docetaxel. These patients were significantly older and had a faster AMH decay rate than those with persistent menstruation at the end of the treatment.
Limitations, reasons for caution: Although calibrated with human recombinant AMH, the ELISA method used suffers from a few technical limitations, such as relatively high functional sensitivity. Only women treated with FECT regimen were included in this analysis. Further analysis will be conducted in all patients, including those receiving other chemotherapy protocols.
Wider implications of the findings: This study brings new insights into the dynamics of chemotherapy-induced alteration of ovarian reserve and paves the way for the study of long-term evolution of AMH after treatment completion. This will help providing guidelines for the evaluation of ovarian reserve before CT onset and improve counselling on fertility preservation strategies.
Trial registration number: This trial has been registered in Clinicaltrials.gov database under the reference NCT01114464.

P-473 Ovarian response to controlled ovarian stimulation for fertility preservation before oncology treatment: A retrospective cohort of 128 patients

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Study question: Should women undergoing ovarian stimulation for fertility preservation expect a lower number of oocytes, compared to patients of a similar age with male-factor infertility?
Summary answer: Ovarian stimulation for fertility preservation, before cancer treatment, results in a similar number of oocytes retrieved compared with male-factor infertility patients of the same age.
What is known already: Several studies have failed to demonstrate a significantly different response to ovarian stimulation between infertility patients and those undergoing fertility preservation before cancer treatment. However, others have found a poorer response. The meta-analysis by Friedler et al in 2011 concluded that there was a poorer response to controlled ovarian stimulation (COS) by oncology patients undergoing fertility preservation treatment compared to patients undergoing COS for infertility treatment. More recently, a large, retrospective analysis by Almog et al showed that, compared to those with infertility, oncology patients responded equally well.
Study design, size, duration: Retrospective analysis cohort study comparing the number of oocytes retrieved by oncology patients (n = 128) undergoing COS for fertility preservation treatment between April 2009 and September 2015, to women undergoing COS for IVF/ICSI treatment for male factor infertility in the same centre and time period (n = 1978).
Participants/materials, setting, methods: All oncology patients treated between April 2009 and September 2015 were included. The control group consisted of patients undergoing COS, during the same time period, for male factor infertility. Yields were plotted against age for both groups along with LMS-derived control centiles. Age-specific Z-scores were derived and compared between groups. Linear regression on squre-root transformed oocyte yield with a cubic spline age covariate was used to estimate the difference in yield between groups.
Main results and the role of chance: The median (IQR) ages in the study and the control group were similar [32(27–34) vs. 32(29–35)]. 62 (48%) patients were diagnosed with breast cancer, 6 (5%) with an ovarian malignancy (including borderline) and 60 (47%) with other malignancies. The cancer patients (after adjusting for age) had a very similar distribution of oocyte yield to the control group (with a mean z-score = −0.03 (95% CI −0.25 to 0.19). There was no significant difference in the number of oocytes retrieved between the groups with 95% confidence intervals excluding differences greater than ~1 egg (mean age-adjusted difference 0.07, 95% CI (−1.0 to −1.2) oocytes). In contrast to previous studies, no significant influence of a cancer diagnosis to the ovarian response after COS was demonstrated. All 6 patients diagnosed with ovarian disease had a poor response, but due to the small number of patients, this did not reach statistical significance. No patients in the study group developed ovarian hyperstimulation syndrome (OHSS).
Limitations, reasons for caution: Limitations, reasons for caution: The study group was treated with a higher dose of gonadotrophins to maximise oocyte yield. The numbers for any particular malignancy or co-morbidity are too small to enable the detection of any sub-groups with poor response.
Wider implications of the findings: This is the largest reported cohort of patients treated in the same centre for fertility preservation before oncology treatment. The ovarian response in such patients is similar to that of controls undergoing treatment for male factor infertility. This information should be reassuring both for the patients and their treating physicians.
Trial registration number: N/A.

P-474 Highly sensitive assessment of neuroblastoma minimal residual disease in testicular tissue using RT-qPCR – A strategy for improving the safety of fertility restoration

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Study question: Improvement of a method to detect minimal residual disease (MRD) of neuroblastoma (NB) in human testicular tissue.
Summary answer: The detection of *PHOX2B* mRNA by quantitative reverse transcriptase polymerase chain reaction (RTqPCR) enables sensitive and specific detection of neuroblastoma cells which may contaminate testis.
What is known already: Cryopreservation of testicular tissue is the only option to preserve fertility in prepubertal boys with NB. NB is the most common extracranial solid tumor in children and affects typically infants and has a high dissemination potential. Testicular tissue may contain malignant cells which could lead to recurrence of the primary disease if the tissue is used after to restore fertility. High levels of *TH* (Tyrosine Hydroxylase), *PHOX2B* (Paired-Like Homeobox 2b) and *DCX* (Doublecortin) transcripts in bone marrow and blood at diagnosis and at the end of induction treatment were recently shown to be poor prognostic factors in NB patients.
Study design, size, duration: Detection of residual disease was performed on fresh and frozen testicular tissues from 20 men with azoospermia between November 2014 and September 2015 by RT-PCR after contamination by increasing amount of two cell lines of NB.
Participants/materials, setting, methods: Written informed consent was obtained for inclusion of any testicular sample from these patients in the Germetheque Biobank (ethical committee DC-2008-558). Frozen testicular tissues by slow freezing (n = 10) with cryoprotectant (DMSO) or by snap freezing in liquid nitrogen (n = 10) were contaminated by increasing amount (0, 10, 100, 1000 cells) of two human neuroblastoma cell lines (IMR-32 and SK-N-SH) before detection of *TH*, PHOX2B and *DCX* transcripts by RTqPCR. All measurements were performed in duplicate.
Main results and the role of chance: *TH* and *DCX* transcripts were detected in uncontaminated testicular tissues. *PHOX2B* was not detected in any uncontaminated testicular fragment (n = 20). We observed a positive correlation between the expression level of *PHOX2B* transcripts and the amount of contaminating cells. The weight of the testicular tissue and the freezing mode (with or without cryoprotectant) had no impact on the expression levels. *PHOX2B* is a reliable marker of NB cells contaminating testicular tissue and can be used for molecular diagnosis of residual disease in testis of prepubertal boys with NB. In contrast, *TH* and *DCX* are expressed in uncontaminated testicular tissue and are therefore unlikely to be useful for performing diagnostics in this setting.
Limitations, reasons for caution: NB transcripts detection confirms the presence of malignant cells, but can not predict their metastatic potential. Nevertheless, it would be unethical to use testicular tissue in which tumor cells are present. Our results need to be confirmed by the analysis of testis providing from boys with NB.
Wider implications of the findings: Our study is the first work to evaluate MRD detection using NB mRNAs in human testicular tissue. These results offer hope in the near future to use testicular tissue without oncological risk in men who survive NB, whose fertility has been jeopardized, and who have benefited from testicular tissue cryopreservation.
Trial registration number: NCT 02400970 (Clinical Trial).

P-475 Male fertility preservation outside reprotoxicant treatments: indications, use rate and ART outcome in 1,966 patients

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Study question: What are the indications and straw utilization rates of male fertility preservation outside reprotoxicant treatments?
Summary answer: The main indications were: risk of semen parameters decline, ejaculation disorders and pre-vasectomy; The overall straw utilization rate was 12.9% of patients.
What is known already: The vast majority of autologous sperm banking activity is performed in patients receiving gonadotoxic treatments (mainly cancer therapy); in large cohorts, the reported straw utilization rate is lower than 10% of patients. To our knowledge, very few data are available about male fertility preservation (FP) outside reprotoxicant treatments (ORT), generally obtained from monocentric and small sample studies.
Study design, size, duration: Multicentric retrospective cohort study in 5 centers of the French national sperm banking network (CECOS). Among the global sperm banking activity (10,508 patients), we included 1,966 patients who banked sperm for FP-ORT between 2000 and 2010. We excluded patients who banked sperm: (i) before gonadotoxic treatment (for cancer or other); (ii) before ART in a context of azoospermia, viral risk, oocyte donation, semen collection failure or personal convenience; (iii) with incomplete data.
Participants/materials, setting, methods: We analyzed: (i) the evolution of FP-ORT indications between 2000 and 2010; (ii) the proportion of FP-ORT indications among the global sperm banking activity. We recorded the straws characteristics, the rate of patients who subsequently used their straw for ART (until 2013), the delay between sperm banking and straw utilization, the number of straws used for ART, ART type and outcome.
Main results and the role of chance: Male FP-ORT increased from 10 to 22% of overall FP indications between 2000 and 2010. The indications of FP-ORT were classified as 6 groups: a) risk of semen parameters decline (RSPD, 59% patients), including sperm concentration fluctuations, severe oligoasthenoteratospermia (OATS) associated to abnormal hormonal and/or genetic examination (Y chromosome microdeletions, Klinefelter syndrome); (b) ejaculation disorders (17%), including spinal cord injury and diabetes; (c) pre vasectomy (14%); (d) surgical risk (6%) (before a surgery at risk for retrograde ejaculation or testicular function); (e) monorchidism (2.4%) (congenital & acquired etiologies); (f) post treatment (1.8%) (antibiotherapy, varicocele surgery).

Between 2000 and 2013, 12.9% (255/1966) of overall patients used their banked sperm for ART treatment (mean ± SD period of banking before use = 1.6 ± 0.6 year): 27% (89/327) of patients with ejaculation disorders, 14% (158/1161) of patients with RSPD, 0.4% (1/275) of patients with vasectomy. ART treatments included 488 cycles: 430 ICSI (88% of cycles), 15 IVF (3%) and 43 IIU (9%). A mean ± SD number of 3.6 (±4.1) straws/patient was used for ART treatments, which only represents 21% (935/4350) of available straws. ART led to an overall pregnancy rate of 28.4%/cycle, and to the birth of 112 children.
Limitations, reasons for caution: As straw utilizations were recorded until 2013, straw utilization rate could be underestimated for the patients who banked sperm in the last years of the study period.
Wider implications of the findings: Increase of FP-ORT indications in daily practice of sperm banking. Utilization rates higher than in cancer patients. Some indications seem particularly justified (ED, RSPD). Low rate of available straws used for ART treatments: what is the ideal amount of straws to bank?
Trial registration number: None

P-476 Telomerase length in granulosa cells and lymphocytes of women recently diagnosed with cancer

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Study question: Is cancer – as a systemic disease – already damaging human ovarian granulosa cells due to telomerase shortening?
Summary answer: The lower ovarian response observed cannot be explained by a telomeric shortening that would suggest a systemic damage of the oncologic disease.
What is known already: The impact of oncologic therapies on fertility outcome is well known, as they may induce irreversible changes in the gonads in at least one third of the patients, affecting future fertility potential. For these reasons, fertility preservation has become a priority for young women with cancer. Interestingly, this group of young women suffering from cancer shows a lower response to ovarian stimulation, even prior to their chemo- or radiotherapy.
Study design, size, duration: Prospective, experimental study.
Participants/materials, setting, methods: we evaluated 24 patients recently diagnosed with cancer that requested fertility preservation and 25 oocyte donors as controls. Ovarian stimulation we performed with recFSH and GnRH antagonist along with letrozole in hormone dependent cancer. Telomeric length was measured in both granulosa cells and peripheral blood (lymphocytes) by quantitative (Q)-FISH, by marking telomeres of interphase nuclei and hybridizing with telomeric repetitions (Cy3).
Main results and the role of chance: We did not observe differences in the telomeric length between cancer patients and controls. When evaluating granulosa cells, mean% of telomere shortening in cancer patients was 13.9 kb (4.7–42.7) whereas in healthy controls was 17.1 kb (4.6–32.8), $p = 0.7$; when peripheral blood was analysed, mean shortening of telomeres was 18.1 kb (4.2–40.6) in cancer patients vs 17.4 kb (6.1–32.3) in healthy patients.
Limitations, reasons for caution: The lack of association between telomere length and ovarian response does not rule out that some of these young women recently diagnosed with cancer do not respond to gonadotropins as expected, may be due to other pathways.
Wider implications of the findings: Although we know today that cancer is a systemic disease with a local origin, we could not confirm that the lower ovarian response to gonadotropin stimulation observed in some women diagnosed with cancer was due to a shorter telomere length, as a reflection of advanced aging due to cancer.
Trial registration number: PI11/02747.

P-477 Transport and handling of oocytes after vitrification in a closed vitrification system (Rapid-i): Proposal for and implementation of a standard validation procedure

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Study question: Can one establish and validate a protocol for handling and transportation of mouse oocytes after vitrification in a closed vitrification system using quality control measures?
Summary answer: Vitrification of oocytes in a closed system and in particular subsequent transportation is safe and reliable but should be shown by a strict validation protocol.
What is known already: Vitrification of metaphase-II-oocytes has opened a new era in the field of fertility preservation. An important aspect of cryobanking of vitrified oocytes is the safety of transportation and handling of vitrified samples. Any increase in the temperature during transportation, storage or handling above a certain threshold will inevitably damage or even destroy oocytes/embryos. This risk an eventually occur when using dry shippers for transportation. Currently there is no international standard regarding the validation of transportation and handling conditions.
Study design, size, duration: After vitrification in a closed vitrification system (Rapid-I, Vitrolife), mouse oocytes were shipped over night to another cryobank, stored in another tank and transported back. Temperature was continuously logged at all stages of handling and transportation. Controls were kept in one cryobank and not shipped. For shipped oocytes and controls the survival after warming as well as fertilization and cleavage rates after ICSI were assessed.
Participants/materials, setting, methods: 16 vitrification experiments were performed, and for each experiment 12 mouse oocytes were vitrified on four Rapid-i straws (Vitrolife, Sweden). Oocytes from 8 vitrification experiments were independently transported back and forth from one cryobank to another using a dry shipper (MVE or Lab-Tec) equipped with temperature logging (Log 100/110 or Testo 176-T4). Oocytes were warmed according to protocol, subjected to ICSI and cultured.
Main results and the role of chance: In eight transportation experiments no adverse events with transportation or handling during loading/unloading of the transportation devices were noted. In all transportation experiments the logged temperature profile was below −150 degrees, which was taken as the highest acceptable limit for the upper temperature. The temperature profile during storage in the receiving cryobank showed a steady storage temperature at −185 degrees or below. After warming of oocytes, survival rates were not different between the control and the study group (90/96, 93.8% and 93/97, 95.9%, respectively). In-vitro fertilization with frozen/thawed mouse sperm resulted in a fertilization rate of 78.1% in the control group and 84.5% in the study group and all oocytes cleaved. Results were not significant for survival and fertilization rates.
Limitations, reasons for caution: This study was performed with mouse oocytes and results need to be validated for human oocytes. Study results only apply to the devices used and other devices may require independent validation by using a similar or adapted protocol.
Wider implications of the findings: Vitrification of oocytes is increasingly performed in human IVF. Although vitrification is accepted as a standard of care for cryopreservation of oocytes, transportation and handling of vitrified oocytes requires special precaution as even minor mistakes can cause complete damage. The validation protocol presented may serve as a standard validation protocol.
Trial registration number: Not applicable.

P-478 Time between exposure to first line of chemotherapy and oocyte retrieval for fertility preservation: cytotoxicity and genotoxicity assay of cytarabin and daunorubicin on mouse ovaries

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Study question: Do cytarabin and daunorubicin induce genotoxicity in mouse oocytes of non-growing and antral follicles exposed, and if so, is oocyte able to repair DNA damage?
Summary answer: Cytarabin and daunorubicin did not induce follicle loss. DNA damage was significant in mature oocytes 6 days and 4 weeks post-exposure but dropped at 12 weeks.
What is known already: Hematological disturbances of acute leukemia and the emergency to start chemotherapy do not allow a cryopreservation of ovarian tissue or oocytes at the time of diagnosis. Cryopreservation of ovarian tissue is currently discussed because of the risk of contamination by leukemic cells and the risk of relapse if reintroduction of malignant cells able to proliferate after ovarian transplantation. In acute leukemia, cryopreservation of mature oocytes after ovarian stimulation could be proposed, but would be performed after a first line of chemotherapy. Genotoxic risks of cytarabin and daunorubicin on oocyte DNA are currently not well known.
Study design, size, duration: Two groups of female CD1 mices aged 4 weeks were exposed for four days to cytarabin ($n = 32$) twice a day (10 mg/kg IP) or every 2 days to daunorubicin ($n = 32$) (1 mg/kg IV). Each group was compared with none-exposed ($n = 32$). In each group, three sub-groups of mice were stimulated respectively 6 days, 4 weeks and 12 weeks post-exposure to retrieve mature oocytes, whole ovaries in order to study chemotherapy effects at different stages of follicle maturation.
Participants/materials, setting, methods: Cytotoxicity was assessed by the number of mature oocytes retrieved, the cytological analysis of oocytes and the

histology of ovaries with quantification of intra-ovarian follicles. DNA damage was assessed by: (*i*) alkaline comet assay to quantify the tail DNA in mature oocytes (*ii*) fluorescent immunohistochemical staining in ovarian tissue sections to quantify accumulating γH2AX foci (expressed as corrected total cryosection fluorescence). The differences between the exposed and non exposed groups were assessed using ANOVA.

Main results and the role of chance: Cytarabin and daunorubicin did not induce a significant intra-ovarian follicular loss and had no impact on ovulation: superovulation was obtained in all mice 6 days, 4 weeks and 12 weeks post exposition and did not differ from control groups. Concerning intra-ovarian follicles, γH2AX foci were significantly increased 6 days, 4 weeks and 12 weeks post exposition compared to none-exposed mice. Binocular analysis did not observe differences of metaphase II oocyte morphology between exposed groups and negative control group. The tail DNA rate was significantly increased in mature oocytes after mice exposure to cytarabin and daunorubicin, compared with the none-exposed 6 days mice (respectively for cytarabin and daunorubicin: 10.7 ± 1.1 and 17.7 ± 1.2 vs. 7 ± 1, $p < 0.0001$) and the non-exposed 4 weeks mice (respectively 15.6 ± 0.8 and 14.2 ± 1 vs. 8 ± 1, $p < 0.0001$. Twelve weeks after cytarabin and daunorubicin exposure, DNA damage was no significantly different in mature oocytes between exposed groups and control group.

Limitations, reasons for caution: Mouse oocyte DNA is not exactly similar to human oocyte DNA, and would be more sensitive to genotoxic effects of chemotherapy agents. The kinetic of oocyte DNA repair post –chemotherapy exposure has to be studied by further assays.

Wider implications of the findings: Despite a good ovulation, DNA damage was observed in mature oocytes 6 days and 4 weeks after exposure to cytarabin or daunorubicin. Twelve weeks post exposure (i.e., after 4 cycles of mouse folliculogenesis), decrease of DNA strand breaks to normal levels let us hypothesis DNA repair mechanisms in non-growing follicles.

Trial registration number: Experimental protocols and animal handling procedures were reviewed and approved by Local Ethics Committee on Animal Experimentation.

P-479 Immature oocyte retrieval followed by *in vitro* maturation (IVM) and vitrification in combination with laparascopic ovarian tissue cryopreservation (OTCP) for fertility preservation: UK pilot study

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Study question: To study the feasibility of oocyte retrieval, *in vitro* maturation (IVM) and subsequent vitrification of mature oocytes in oncology patients undergoing ovarian tissue cryopreservation (OTCP) for fertility preservation

Summary answer: The pilot study revealed that IVM of immature oocytes followed by vitrification could be successfully achieved in some oncology patients undergoing OTCP

What is known already: Previous studies in Europe, USA and Israel have already shown that the combination of ovarian tissue cryopreservation and immature oocyte retrieval followed by vitrification is a feasible option, irrespective of the phase of menstrual cycle or age of the patient. This is the first time this approach has been attempted in the UK in both paediatric and adult patients to try to maximise fertility preservation with a patient as young as 2 years of age.

Study design, size, duration: Prospective cohort pilot study from 2013 to 2015 of 23 oncology patients, both paediatric and adult, age range 2–31. Laparoscopic immature oocyte retrieval and removal of ovarian tissue for cryopreservation was scheduled at the time of insertion of the Hickman line, to avoid the patient undergoing multiple procedures.

Participants/materials, setting, methods: Patients undergoing laparoscopic ovarian tissue harvesting before cancer therapy were offered immature oocyte retrieval. Oocytes were retrieved by three methods: *in situ* percutaneous video-assisted oocyte retrieval for those patients undergoing ovarian cortical strip resection (rather than oophorectomy), *ex-situ* puncturing of the excised ovary and removal of the fluid remnants post dissection and tissue processing of the ovarian tissue. Oocytes were then matured *in vitro* using standard IVM methodologies and any resultant mature oocytes vitrified.

Main results and the role of chance: Of the 23 patients who participated in the pilot study, 15/23 (65%) were paediatric (age 2–17) and 8/23(35%) adults (age 22–31). Successful immature oocyte retrieval was achieved in 78% of the patients, 80% in the paediatric v 75% in the adult group, with the majority of the oocytes being isolated from the dissection remnants (61%). A total of 140 oocytes were collected, of which 57 (41%) were degenerate and therefore non-viable. Of the remaining 83 oocytes, 40 reached metaphase (MII), giving a maturation rate of 48% following 24–48 h in IVM culture. The remaining 43 (52%) arrested at germinal vesicle (GV) stage. All metaphase II oocytes were subsequently vitrified. Vitrification of mature oocytes was achieved in 60%(9/15) of the paediatric patients (range 1–5), the youngest of which was aged 2 (1 frozen), which to the best of our knowledge is the youngest patient to date, in comparison to 38% (3/8) in the adult group.

Limitations, reasons for caution: None of the oocytes vitrified in this study have been thawed and therefore their viability remains unknown. Also, due to restrictions in both the timings and collection methods of the oocytes the quality of the oocytes may be compromised as the main priority was OTCP.

Wider implications of the findings: This pilot study showed that immature oocytes can be successfully harvested/matured and vitrified from both antral follicles and excised tissue in both paediatric and adult patients undergoing OTCP. This is in accordance with other published studies. However, this study has also demonstrated freezing for the youngest patient to date.

Trial registration number: Not applicable.

P-480 Oncofertility: Optimum transport conditions for ovarian tissue cryopreservation prior freezing

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Study question: Which is the optimal transport temperature for ovarian tissue and how long could the tissue be transported prior cryopreservation without disturbing viability?

Summary answer: This study provides evidence that storage at 4°C is superior to warm transport (38°C). Storage duration should be limited to a minimum to prevent potential damage.

What is known already: Cryobanking with transportation of ovarian cortex before cryopreservation is clinically used to make the option accessible to patients in regions in which a fertility centre with the required facilities and skills is not available. It is a useful option for patients with serious illnesses in whom the start of treatment cannot be postponed and who therefore may not wish to move from one center to another, or may not be able to. However data about transport conditions of ovarian tissue in general are limited. Further investigations on this field are necessary immediately to clearly guarantee the ovarian tissue viability.

Study design, size, duration: The aim of this prospective controlled animal study was to evaluate the rate of follicle loss over various time periods within temperature treatment of 4°C and 39°C and to assess whether ovarian follicle viability is affected following cryopreservation and thaw subsequent to the transportation of ovarian tissue by using markers of tissue viability. To address this aims, pig ovaries were utilized because pigs have been demonstrated to be an appropriate model for human reproductive biology.

Participants/materials, setting, methods: Pig ovaries were transported either at 4°C ($n = 20$) or at 38°C ($n = 20$) prior to cryopreservation. At 0 h, 4 h, 12 h and 24h ovarian tissue (same size $6 \times 4 \times 1$ mm) were fixed for histological examination. At the same time point's ovarian tissue were cryopreserved with a slow-freezing procedure and analyzed after thawing. Furthermore viability testing in SCID mouse transplantation model ($n = 120$) and a LIVE-DEAD assay were performed.

Main results and the role of chance: Histological evaluation showed an intact morphology in the fresh and cryopreserved ovarian tissue pieces at 4°C and at 39°C over the transportation time. The follicle reserve (primordial and primary follicles) decreased significantly in a time-dependent manner. The follicles in the cryopreservated ovarian tissue decreased statistically significantly in both storage groups compared with fresh ovarian tissue. At 39°C a significantly higher follicle loss over the transport duration was observed in comparison with 4°C ($p < 0.018$). At 4°C temperature, the Live Dead assay showed that the number of viable follicles remained approximately constant over the transport time up to 24 h (mean: 78% live cells). At 39°C the number of viable follicles was significantly reduced after 24 h (mean: 27% live cells) compared to the time point 0 h (mean: 82% live cells). Morphological quality and the number of follicles decreased in ovarian tissue that had been xenotransplanted in SCID mice in a time-dependent manner and were even poorer

if the tissue was cryopreserved after storage in comparison to fresh stored ovarian tissue. At 4°C follicle atresia was not significant over the transportation time, however at 39°C a significant follicle loss after 12 h transportation could be detected ($p < 0.4$).

Limitations, reasons for caution: In this study pig ovarian tissue was used. Further studies with human ovarian tissue need to evaluate the effect of temperature, duration and media during transportation and establish the present method as a full - scale clinical application. Since ovarian cryopreservation and transportation is increasingly used, further studies are urgently needed.

Wider implications of the findings: Prolonged tissue transportation must not be considered a routine and transportation durations should be kept at a minimum to prevent potential damage. In individual cases, where otherwise ovarian tissue cryopreservation could not be offered, prolonged transportation at 4°C could be performed, as the follicle loss is modest as shown.

Trial registration number: None.

P-481 Fertility preservation public program. Is it worthwhile?

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Study question: To evaluate first three yers of development of a fertility preservation (FP) public program and to analise the impact of the formation activities towards professionals who attend oncologycal patients.

Summary answer: Patients with malignant disease who undergoing FP techniques have risen.

There are important gender disparities in FP.

Information from health care providers is essential.

What is known already: For many young cancer survivors, the ability to have biological children is a significant goal and concern. FP has being used routinely for relatively few years and there is still a gap in knowledge about the performance of the technique in the future when female patients come back to use their oocytes. Vast majority of men use sper banking.

Despite the ASCO guidelines, many cancer patients do not receive enough information about FP.

On the other hand, females paid more for FP than males.

Study design, size, duration: Observational and retrospective study from 2013 to 2015 in a Spanish region over 1 million of habitants.

Male and female patients who attended for FP are included. All cases have medical indications (oncological and no oncological) for FP.

Participants/materials, setting, methods: A total of 69 male and female patients were enrolled in this observational study since we started the FP program three years ago.

Patients were submitted from oncologist or other specialist to Human Reproduction Unit.

We analyse the follow questions: number of patients who receive information for FP, number of patients who undergo FP techniques, techniques employed and number of patients that return to use their gametes.

Main results and the role of chance: From 2013 to 2015, we attended 69 patients (20 women and 49 men) who asked about FP options. Most prevalent female indication were breast cancer (64.28%) and Hodgkin disease (14.28%). Male indications were testicular tumors (61.22%), hematological diseases (36%) and chromosomal disorders (2.04%).

62 of all of them (89.85%) opted to undergo treatment. There were significant differences between female (70%) and male (98%) percentages. Women's reasons for not undergoing FP were lack of time to complete controlled ovarian hyperstimulation and lack of oncologist's agreement. Techniques employed were oocyte vitrification as first option or embryo preservation (if there was low number of oocytes and partner's agreement). Ovarian cortex preservation and ovarian transplantation are not legally allowed in our hospital.

All of men used sperm banking.

So far, no one, male or female, has returned to use their gametes or embryos.

Limitations, reasons for caution: Not enough time has elapsed to evaluate the cost effectives results of FP techniques, but the simple fact of undergoing FP improves the patient's experience with oncological treatments. Future studies could examine the reasons physicians are less likely to adress FP with women (higher costs and the time needed).

Wider implications of the findings: We should inform oncologists about referring patients to specialists in FP in a timely manner.

FP for oncological reasons seems out of question, but it needs to be clearly specified which women may benefit from FP. Recommendations should be individualized.

More studies are needed to evaluate results (new borns) from FP techniques.

Trial registration number: Present study is not a clinical trial.

P-482 Topical high concentration of anti-Müllerian hormone prevents follicle activation and loss caused by chemotherapy *in vivo*

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Study question: Does anti-Müllerian hormone (AMH) prevent cyclophosphamide (Cy)-induced follicle activation and loss?

Summary answer: AMH prevents follicle activation and loss caused by Cy in mice.

What is known already: Chemotherapeutic regimens can damage the reproductive system and lead to infertility and premature ovarian failure. AMH, which is produced by the granulosa cells of growing follicles, can inhibit primordial follicle activation and follicle growth stimulated by follicle-stimulating hormone.

Study design, size, duration: It was an *in vivo* study, in which twenty-four mice were randomly divided into three groups: control ($n = 6$), Cy ($n = 6$) and AMH + Cy ($n = 12$). Examinations of ovaries were performed 7 days later.

Participants/materials, setting, methods: In the Cy group, six seven-week old ICR female mice treated intraperitoneally with Cy (150 mg/kg). Mice in the AMH + Cy group received intrabursal injection of low-dose recombinant AMH (1 μg/mL, left ovary) and high-dose recombinant AMH (10 μg/mL, right ovary), followed by Cy treatment 1 h later. Follicle counting, immunohistochemical analyses and mRNA levels of follicle stage-specific markers were performed after removal of ovaries.

Main results and the role of chance: In the Cy-treated ovaries, the number of primordial follicles was decreased significantly. Mice pretreated with recombinant AMH had more primordial follicles than those in the Cy group ($P < 0.05$) with dose-dependent effects. The results were compatible with the mRNA levels of Lhx8 and Nobox, indicating that recombinant AMH prevented the recruitment of follicles triggered by Cy.

Limitations, reasons for caution: The inconsistency between models might reflect different ovarian responses to different manipulations. The best dosage and time to treatment and examinations were still undetermined.

Wider implications of the findings: We first demonstrated that pretreatment with topical high concentration of recombinant AMH inhibited follicle activation and preserved more follicles *in vivo*. This preliminary report showed its therapeutic potential in oncofertility. The protective efficacy still needs more investigation.

Trial registration number: Not available.

P-483 Novel extracellular-like matrices and leukemia inhibiting factor for the *in vitro* culture of human primordial follicles

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Study question: Will novel culture matrices and leukemia inhibiting factor (LIF) improve development of human primordial follicles?
Summary answer: Human recombinant pure plant-line bioengineered collagen (Collage™) and alginate scaffolds seem to marginally improve culture results. LIF does not promote growth of human primordial follicles.
What is known already: Human recombinant vitronectin (hrVit), a matrix protein, promoted proliferation of human embryonic stem cells. Small intestine submucosa (SIS) (Cook Biotechnology), is a natural polymer that promoted growth of various cell types. Human recombinant collagen from bioengineered tobacco plant lines (Collage) does not pose any animal-derived pathogen hazards, and promoted proliferation of various cell types. Alginate scaffolds are biocompatible, allow efficient transport between cells, and have been used successfully to grow various cell types including human ovarian primordial follicles.

LIF increased growth of primordial follicles from rodents and goats. LIF and its receptors were detected in preantral follicles from women.
Study design, size, duration: Frozen-thawed human ovarian samples from 28 women/girls were utilized for the study. In the first part, tissue was cultured on hrVit and on SIS with: basic culture medium (α minimal essential medium + human serum albumin + insulin, transferrin, selenium + FSH) alone, basic culture medium + LIF or + LIF + anti-LIF neutralising antibody. In the second part, tissue was cultured on Collage and on alginate scaffolds with basic culture medium alone. Samples were cultured for six days in both parts of the study.
Participants/materials, setting, methods: Ovarian tissue was obtained from 28 women/girls who underwent ovarian cryopreservation in a tertiary medical center. Initially, tissue was cultured on hrVit and on SIS with: basic culture medium, basic culture medium + LIF (10 and 100 ng/ml) or basic culture medium + LIF (10 ng/ml) + anti-LIF antibody (1 mg/ml). Thereafter, tissue was cultured on Collage and on alginate scaffolds with basic culture medium alone. Growth was evaluated by follicular counts and classification, Ki67 immunohistochemistry and 17β-estradiol (E_2) and anti-Mullerian hormone (AMH) measurements.
Main results and the role of chance: There were significantly more Ki67-stained follicles on Collage and alginate than on hrVit and SIS, without LIF supplementation ($P < 0.0001$). E_2 levels were significantly higher after culture on Collage and alginate than on hrVit and SIS, without LIF supplementation ($P < 0.0003$).

The rest of the comparisons were nonsignificant. In the first part, samples incubated with basic culture medium had a higher developing (primary and secondary) follicle rate than primordial follicles, regardless of matrix (NS). Samples cultured on SIS or hrVit with LIF (both concentrations) and LIF + anti-LIF had a higher atretic follicle rate than samples cultured with basic medium alone, regardless of matrix (NS). Moreover, samples cultured on SIS or hrVit with LIF (both concentrations) and LIF + anti-LIF had a lower follicle rate than samples cultured with basic medium (NS), regardless of matrix. In the second part, there were more atretic follicles ($P = 0.06$), but also developing follicles in samples cultured on alginate than on Collage (NS). The differences in AMH secretion from follicles by culture matrix or by LIF supplementation were nonsignificant.
Role of chance: There is a variability in follicular distribution in human ovaries, which might have contributed to the initial low follicular content in cultured slices resulting in nonsignificant results.
Limitations, reasons for caution: There is a limited availability of human tissue for research. Therefore, only seven samples were used in parallel in both study parts. The variability in the initial follicular content between the different samples (between individual patients and between different samples from each patient) might have resulted in mostly nonsignificant results.
Wider implications of the findings: Unlike findings in other mammals, growth of human primordial follicles is not enhanced by LIF. Collage and alginate seem associated with marginally improved results than SIS and hrVit. Further studies should be conducted to investigate other matrices and additional growth factors and substances for growth promotion of human primordial follicles.
Trial registration number: This not a clinical study. Therefore, there is no trial registration number.

P-484 The effects of the cancer drug Cyclophosphamide on mouse fertility

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Study question: To examine the effects of a cancer drug on ovarian function and maternal behavior
Summary answer: Cancer drugs negatively affect ovarian function (up to 2 month post-administration) and maternal behavior.
What is known already: According to studies on female rats, the teratogenetic action of fetus or embryonic death has been reported when cyclophosphamide has been administered.
Study design, size, duration: Study1: This is a cross-sectional, longitudinal study in which female mice were treated one time with a cancer drug and then mated: 0 h group $n = 10$; 2 weeks (post drug treatment) $n = 10$; 1 month $n = 16$; and 2 month $n = 2$, (control $n = 11$).

Study2: A cross-fostering experiment in which 7 female mice were treated with a cancer drug and immediately mated. The treated mothers and the 7 control mothers were switched to care for each other's pups.
Participants/materials, setting, methods: Study1: All mice used in these studies were 8 week old ICR mice. 38 female mice at 8 weeks old were treated with a single injection of cyclophosphamide (400 mg/kg intraperitoneally). The control group consisted of 11 mice treated with a single injection of saline. Study2: 400 mg/kg administered once to 7 mice, control group ($n = 7$) did not receive drug. Control group mated. Treated group mated. Control and treated mothers switched at post natal day 2.
Main results and the role of chance: Study1: The survival rate of infants in the Cyclophosphamide group at day 20 was significantly lower than the control group. However, though the sample size is currently limited ($n = 2$), preliminary results are positive with the test group survival rate dramatically increasing when measured at 2 months post drug treatment. Study2: The survival rate of the pups significantly increased when the cyclophosphamide treated mother was replaced with the mother of the control group. Conversely, the survival rate significantly decreased when the cyclophosphamide administered mother raised the pups born from the control group.
Limitations, reasons for caution: The study was limited to one kind of cancer drug, administered once, and 1 mouse strain. Moreover, the study population is small, but the study is ongoing.
Wider implications of the findings: These preliminary date suggest that the cancer drug affects mothers' behavior while not having strong effects on oocyte quality.
Trial registration number: Not applicable.

P-485 Antral follicle responsiveness to exogenous FSH assessed by the Follicular Output RaTe (FORT) is altered in Hodgkin lymphoma patients in comparison with breast cancer

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Study question: To investigate whether responsiveness of antral follicle to exogenous FSH, assessed by the Follicular Output RaTe (FORT), differs between Hodgkin lymphoma and breast cancer patients?

Summary answer: Antral follicle responsiveness to exogenous FSH assessed by the Follicular Output RaTe (FORT) is altered in Hodgkin lymphoma patients in comparison with breast cancer.
What is known already: Oocyte and/or embryo cryopreservation after controlled ovarian hyperstimulation (COH) represents the most established method of fertility preservation (FP) before cancer treatment. Several line of evidence indicate that patients suffering from malignancies may be less prone to respond to ovarian stimulation, possibly as a result of a reduced antral follicle endowment. The percentage of antral follicles that successfully respond to FSH administration (FORT), which presumably reflects the health of granulosa cells and follicle quality, may differ according to the type of malignancy.
Study design, size, duration: From July 2013 to April 2015, we prospectively studied 80 cancer patients, 20–40 years of age, candidates for FP using oocyte and/or embryo vitrification following COH. Of these, 58 were suffering from breast cancer and 24 had Hodgkin lymphoma.
Participants/materials, setting, methods: All women had 2 ovaries, no history of chemotherapy, and underwent COH using GnRH antagonist protocols. Evaluation of the follicular ovarian status by measurement of serum anti-Müllerian (AMH) levels and antral follicle count (AFC) was systematically performed before exogenous FSH administration. FORT was determined by the ratio between the pre-ovulatory follicle count (PFC, 16–20 mm) on the day of oocyte triggering (dOT) × 100/AFC measured just before initiation of the stimulation (d0).
Main results and the role of chance: Overall, women in the Hodgkin lymphoma group were younger in comparison with breast cancer patients (26.7 ± 3.7 vs 33.6 ± 3.9 years, $p < 0.0001$, respectively). Although d0 AFC was significantly higher in women suffering from hemopathy (24 ± 13 vs. 19 ± 14 follicles, $p < 0.02$), serum AMH levels remain similar in both groups (3.7 ± 2.7 vs. 3.3 ± 3.4 ng/mL, *NS*). Interestingly, the FORT was significantly decreased in patients with Hodgkin lymphoma when compared with breast cancer group (25 ± 16 vs 37 ± 19%, $p < 0.02$, respectively), leading in the end to a comparable number of oocytes vitrified (9.9 ± 5.6 vs. 9.4 ± 7.2 oocytes, *NS*, respectively).
Limitations, reasons for caution: FORT presents inherent limitations. Indeed, FORT calculation assumes that small antral follicles, respond in a coordinated manner to FSH and that only follicles having reached diameters ranging between 16 and 20 mm on dOT effectively responded to FSH. In addition, the sample size may limit the generalization of these findings.
Wider implications of the findings: The present findings indicate that the percentage of antral follicles that successfully respond to FSH administration (FORT) is reduced in Hodgkin lymphoma when compared to breast cancer patients, supporting the hypothesis of a detrimental effect of the hemopathy on follicular quality.
Trial registration number: No.

P-486 Exploring the fertility potential of GV-retrieved oocytes for future fertility preservation

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Study question: What is the fertility preservation (FP) potential of GV-retrieved oocytes after denudation, *in vitro* maturation (IVM), vitrification and ICSI?
Summary answer: Seventy percent of GV to MII stage IVM oocytes were fertilized and half of these developed into cleavage stage embryos.
What is known already: In clinical programs for fertility preservation, following conventional ovarian stimulation regimens and follicular aspiration, approximately 15–20% of oocytes retrieved are found at an immature stage upon denudation, prior to vitrification. IVM of such immature oocytes has been proposed as an additional method of fertility preservation to increase the yield of mature oocytes which can later be thawed and fertilized by ICSI.
Study design, size, duration: A step-wise prospective randomized study in an academic reproductive medicine center. Power calculation estimated a need of 50 GV oocytes per group to identify an optimal culture media setting in step I. In step II, IVM matured oocytes were randomly assigned to either immediate vitrification or fresh ICSI using cryopreserved and thawed sperm from a single donor, followed by fertilization check after 16–20 h and subsequent embryo culture. The study is still ongoing.
Participants/materials, setting, methods: The study was approved by the regional ethics committee. Immature oocytes were donated to research from infertile women upon undergoing IVF-ICSI treatments, following written consent. In step I, a total of 100 denuded GV oocytes were randomized to either one of two commercially available IVM culture media. The identified optimal culture setting was applied in step II, where all procedures were performed by one of 2 experienced embryologists in a routine clinical embryology laboratory setting.
Main results and the role of chance: One of the two media culture used was identified as superior, as it resulted in significantly higher numbers of GV oocytes reaching MII stage after 24 h of culture, 85.7% vs. 41.2% ($P < 0.001$). No further improvement was observed after longer culture periods. Up to date, a total of 80 oocytes have been treated by ICSI after IVM with a fertilization rate of 70.6%. However, about only half of those have further developed to a cleavage stage embryo.
Limitations, reasons for caution: There is a substantial lack of published data regarding the outcome of GV-obtained oocytes that undergo IVM and the potential use of these for cancer patients undergoing FP by ovarian stimulation/oocyte retrieval prior to initiation of gonadotoxic chemotherapy.
Wider implications of the findings: IVM may improve the yield of mature oocytes for women undergoing ovarian stimulation/oocyte retrieval FP. However, the efficacy and safety aspects of IVM in combination with vitrification and subsequent ICSI requires further investigation.
Trial registration number: N/A.

P-487 Moues fresh and vitrified ovarian tissue transplantation under influence of the static magnetic field

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Study question: Does the static magnetic field (SMF) influence in transplantation of fresh and vitrified ovarian tissues into the testis?
Summary answer: SMF with moderate intensity has been shown to be effective on biological systems also it is shown to be an effective method for ovarian transplantation.
What is known already: Ovarian tissue banking is a promising option for preserving fecundity in young female cancer patients facing sterilization by chemotherapy and/or radiotherapy. However, the limited functional duration of some non-vascularized ovarian grafts may be due in part to ischemic injury sustained until revascularization is adequate. Many researchers attempted to find a way for minimizing the length of time which tissue must spend in an ischemic state after ovarian transplantation.
Study design, size, duration: The SMF strength in all experiments was 1 mT and it applied for 10 min. In this study used from 6 to 8 week-old female Naval Medical Research Institute (NMRI) mice. Morphological evaluations of ovaries and molecular analysis for active-caspase 3 apoptotic protein and CD31 angiogenic molecule were performed next 3 weeks of transplantation.

To understand follicular and oocyte development was investigated the endocrine function of grafts in male mice receiving ovarian.
Participants/materials, setting, methods: Ovaries of mice randomly were divided into 4 groups: (1) fresh ovaries were immediately transplanted into testicular tissue (FOT group), (2) fresh ovaries were exposed to the SMF for 10 min then were transplanted into the testicular tissue (FOT^+ group), (3) vitrified-warmed ovaries were transplanted into the testicular tissue (VOT group) and (4) vitrified-warmed ovaries were transplanted into the testicular tissue then transplantation site were exposed to a SMF for 10 min (VOT^+ group).
Main results and the role of chance: data indicate that the lowest percentages of morphological dead primordial follicles and the highest percentages of morphological intact primordial follicles were seen in FOT^+ group (4.11% ± 2.88 and 41.26% ± 0.54) respectively. The lowest percentage of intact primordial follicles was seen in the VOT group (24.82% ± 1.03). Alteration in the number of blood vessels had a clear difference between FOT^+ (18.60 ± 0/51) and VOT (7.80 ± 0/58) groups but their results in existence of blood vessels were not significant against the other groups. There was not a statistically difference between FOT, FOT^+, and VOT^+ groups in terms of apoptotic rate and number of newly formed blood vessels.

The hormonal assay showed that the level of plasma progesterone increased in FOT+ group in compared to other groups. Also In grafted male mice, estrogen (E2) and progesterone (P4) concentrations were significantly higher than of the control mice. The level of testosterone (T4), in control group was analytically higher than the other experimental groups.

Limitations, reasons for caution: Since human ovarian tissue is scarce, the present protocol was developed in the mouse model. Further studies are necessary to determine the exact mechanism of SMF effects on human ovarian transplantation.

Wider implications of the findings: An optimal transplantation protocol enhances the chances for successful fertility restoration. Exposure of mice ovarian tissues to static magnetic field before transplantation resulted in greater transplantation outcomes against non-exposed grafted tissues. Exposure of the vitrified-warmed ovaries to SMF led to maintain the structure of the graft similar to fresh ovaries.

Trial registration number: NA.

P-488 Administration of a GnRH agonist during chemotherapy for breast cancer reduces ovarian toxicity in women aged under 40 years

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Study question: Does administration of a GnRH agonist during chemotherapy for breast cancer reduce ovarian toxicity, as reflected in the prevalence of ovarian failure?

Summary answer: Administration of goserelin during chemotherapy reduced the likelihood of ovarian failure at 2 years after randomisation, and this was most apparent in women aged ≤40.

What is known already: Chemotherapy can cause ovarian toxicity, usually determined as ovarian failure with amenorrhoea, with lesser degrees of damage resulting in incomplete loss of ovarian reserve. Whether administration of a GnRH agonist during chemotherapy for breast and other cancers can reduce this has been controversial for many years. Although recent RCTs have indicated that there may be a benefit, the degree of ovarian protection afforded and the applicability across all age groups remain unclear.

Study design, size, duration: OPTION' is a prospective, multicentre open parallel group RCT of the administration of goserelin vs no treatment in premenopausal women with early or locally advanced breast cancer where adjuvant or neo-adjuvant chemotherapy was indicated but ovarian suppression was not. We report the results for ovarian failure at 2 years after randomisation, defined as amenorrhoea between 12 and 24 months, supported by hormone measurements. Planned recruitment was 250 patients over 5 years, with stratification by age.

Participants/materials, setting, methods: Premenopausal women were randomised by a central trial centre to goserelin 3.6 mg sc starting 1-2 weeks before chemotherapy and continuing every 4 weeks until the end of chemotherapy, vs. no treatment. Chemotherapy included 6–8 cycles of cyclophosphamide and/or anthracycline-containing regimens. Patients were followed-up 6-monthly for 2 years. Hormone levels were determined pretreatment and at intervals to 2 years, during which time a menstruation diary was also kept.

Main results and the role of chance: 227 patients were randomized, 3 in each arm were omitted from analysis because they had died and a further 19 patients (11 in the control arm and 8 in the goserelin arm), because menstrual status between 12 and 24 months could not be determined. Women in the goserelin arm were significantly more likely to have amenorrhoea: 12% vs. 38% ($p = 0.015$, $n = 202$); this difference was more robust when a criterion of high FSH was added. Two alternative imputations were made assuming all 19 women with missing data either did or did not have ovarian failure: in both cases there remained a significant benefit of goserelin. Stratification by age indicated a beneficial effect from goserelin in women aged 40 or less (10% vs. 25% with menses, $p = 0.032$, $n = 119$) which was not apparent in those over 40 (43% vs. 57%, $p = 0.38$, $n = 83$). Anti-Mullerian hormone (AMH) was available in a subset of women; this did not show significant differences between treatment groups but highlighted the marked impact of chemotherapy, with a mean reduction of 76% vs pretreatment overall, and of 70% in those with ongoing ovarian function.

Limitations, reasons for caution: These data do not explore fertility or other long-term health outcomes. The AMH data indicate that the extent of preservation of ovarian function is limited compared to the amount lost from chemotherapy. It is unknown whether these results can be translated to other diagnoses or chemotherapy regimens.

Wider implications of the findings: The preservation of ovarian activity in some women may have implications for quality of life and longer-term health, such as fertility, and bone and cardiovascular health.

Trial registration number: EudraCT 2004-000133-11.

P-489 Comparison of GnRH agonist (GnRHa) and hCG for priming before *in vitro* maturation (IVM) and oocyte vitrification in cancer patients undergoing urgent fertility preservation (FP)

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Study question: To compare the number of metaphase 2 (M2) oocytes vitrified following IVM in candidates for urgent FP having received a GnRHa or an hCG priming.

Summary answer: GnRHa priming significantly increases the number of cumulus-oocyte-complexes (COC) recovered but not the number of M2 oocytes available for cryopreservation when compared to hCG priming.

What is known already: IVM of oocytes retrieved at germinal vesicle stage, followed by vitrification of M2 oocytes, has recently emerged as an option for young women seeking urgent FP. This technique was originally developed for patients with polycystic ovary syndrome, in whom the induction of a LH surge, provided with hCG administration, 36 h before COC retrieval, improved outcomes. Evidence indicates that ovulation may be efficiently triggered in controlled ovarian stimulation cycles by administering a combination of FSH + LH activity using GnRHa. Whether GnRHa administration impacts on immature oocyte retrieval and their capacity to mature *in vitro* is not established.

Study design, size, duration: From January 2009 to April 2015, 436 cancer patients, 18–40 years of age, candidates for urgent FP, were prospectively studied. All women underwent M2 oocyte cryopreservation after IVM cycles primed either with GnRHa (triptorelin 0.2 mg, SC, $n = 276$) or hCG (Gonadotrophine Chorionic Endo, 10,000 IU, IM, $n = 160$).

Participants/materials, setting, methods: All participants were diagnosed with cancer and underwent FP using IVM. Inclusion criteria were: age 18–40 years; presence of two ovaries; no history of chemotherapy. Before urgent immature oocyte retrieval, follicles measuring 2–9 mm in diameter were counted on both ovaries and serum anti-Müllerian hormone (AMH) was measured, irrespective of the phase of the cycle. Primary outcomes were the number of COC retrieved, the maturation rate and the number of M2 oocytes cryopreserved.

Main results and the role of chance: Overall, patients in the GnRHa and the hCG groups were comparable in terms of age (31.6 ± 4.4 vs. 31.9 ± 4.9 years, respectively, $p = 0.4$), BMI (23.1 ± 4.2 vs. 22.8 ± 3.9 Kg/m^2, respectively, $p = 0.5$) and ovarian reserve tests (antral follicle count: 22.6 ± 14 vs. 20.8 ± 11.9 follicles, $p = 0.2$; serum AMH levels: 4.4 ± 3.9 vs. 4.3 ± 3.5 ng/mL, $p = 0.8$, respectively). The number of COC retrieved was significantly higher in the GnRHa group when compared with hCG group (10.8 ± 9.9 vs. 8.4 ± 6.7 oocytes, $p < 0.008$). Despite a trend to a reduced maturation rate after GnRHa priming (59.1 ± 24.1 vs. $63.7 \pm 20.2\%$, $p = 0.07$), the total number of M2 oocytes frozen was similar in both groups (5.9 ± 5.1 vs. 5.1 ± 4.6, $p = 0.1$, respectively).

Limitations, reasons for caution: The main limitation of our investigation is the absence of randomization. In addition, although oocyte cryopreservation following IVM is a promising method for urgent cases of FP, the true competence of these oocytes remains still ill-established.

Wider implications of the findings: Our findings confirm the capacity of a combined FSH + LH priming, provided by GnRHa administration, to potentially improve IVM cycle outcomes in cancer patients. Randomized control trials

are needed to objectively assess the need for priming in normo-ovulatory cancer patients, and the best way to provide it if required.
Trial registration number: N/A.

P-490 Effects of slow-freezing and vitrification on quality of human spermatozoa

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Study question: Is there an advantage of vitrifies sperms compared with slow-freezing technique regarding motility, survival rate, velocity parameters and acrosome reaction (AR) of human spermatozoa?
Summary answer: All velocity parameters were significantly higher after vitrification compared with the conventional slow-frozen spermatozoa. Higher proportion of AR could be observed in both cryopreserved groups.
What is known already: Cryoreservation of human spermatozoa is widely used in assisted reproduction. It is still under debate for cryopreservation of human semen whether the vitrification technique is superior to the slow-freezing technique or not. Isachenko (2012) already showed that vitrification of human spermatozoa is possible without permeable cryoprotectants to avoid the negative influence by their toxicity. Nevertheless several studies show contradictory results comparing both techniques with respect to different parameters, e.g., motility, viability, DNA integrity and many more.
Study design, size, duration: Human semen samples (n = 50) were obtained from patient donors as a part of their clinical evaluation for reproductive health status collected in three month. All donors practiced 3–5 days of sexual abstinence and their average age was 37.5 ± 6.4 years. Semen analysis was carried out in accordance with the WHO guidelines. Semen samples with a concentration of ≥20 million/ml were used.
Participants/materials, setting, methods: Each density gradient prepared sample was divided into three equal parts: (1) conventional slow-freezing (2) vitrification and (3) control without cryopreservation. The motility parameters that computed and recorded by CASA include progressive and non-progressive motility, vitality, curvilinear velocity (VLC), average path velocity (VAP), and amplitude of lateral head displacement (ALH). Chlortetracycline staining (CTC) was used to evaluate the acrosomal status (AR). The parameters were analysed using a one way ANOVA.
Main results and the role of chance: A one way ANOVA revealed a significantly higher curvilinear velocity (VLC), average path velocity (VAP) and amplitude of lateral head displacement (ALH) of spermatozoa after vitrification compared to the recorded measurements of slow-frozen spermatozoa ($p \leq 0.001$). A Higher but not significantly progressive motility rate (M = 16.18, SD ± 8.12 vs. M = 14.56, SD ± 7.74) and vitality rate (M = 28.36, SD ± 9.03 vs. M = 25.94, SD ± 8.24) could be observed after vitrification compared with slow freezing sperms. No significant difference between the cryopreserved groups could be found in respect to acrosome reaction. However, both cryopreserved groups show significantly higher levels of acrosome reacting ($p \leq 0.001$) and reacted ($p \leq 0.05$) spermatozoa than spermatozoa that did not undergo any cryopreservation.
Limitations, reasons for caution: In the study were oligo-, astheno-, terato- and normozoospermic samples included and only samples with a concentration of ≥20 million/ml after preparation were used. It is possible that for special pathologic findings different cryopreservation methods are superior to others.
Wider implications of the findings: Results described in the present study suggest that motility and vitality of human sperms did not differ after slow freezing and vitrification. According to McLaughlin (1993), no differences in acrosomal status occurred in both groups. However, vitrified spermatozoa show higher velocity parameter levels which are good predictors of fertilizing ability.
Trial registration number: None.

P-491 Letrozole co-administration does not alter antral follicle responsiveness to exogenous FSH assessed by the Follicular Output RaTe (FORT) in breast cancer patients seeking fertility preservation.

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Study question: To investigate whether letrozole co-administration alters antral follicle responsiveness to exogenous FSH, assessed by the FORT in breast cancer patients undergoing ovarian stimulation for fertility preservation?
Summary answer: The percentage of antral follicles that successfully respond to FSH administration remains comparable in breast cancer patients undergoing conventional ovarian stimulation or protocol using letrozole.
What is known already: Oocyte and/or embryo vitrification after controlled ovarian hyperstimulation (COH) represents the most established and efficient method of fertility preservation before cancer treatment. However, traditional COH regimens are associated with supraphysiologic levels of estrogen and as a result are not recommended for breast cancer patients. To protect the patients from the potential deleterious effects of elevated estrogen levels during ovarian stimulation for fertility preservation, protocols using aromatase inhibitors were developed. However, whether the percentage of antral follicles that successfully respond to FSH administration (FORT) differs between conventional and letrozole supplemented protocols remains not established.
Study design, size, duration: From July 2013 to April 2015, we prospectively studied 91 breast cancer patients, 25–40 years of age, candidates for FP using oocyte and/or embryo vitrification following COH. Of these, 37 had letrozole supplementation during COH while 54 underwent traditional GnRH antagonist protocol.
Participants/materials, setting, methods: All women had 2 ovaries, no history of chemotherapy, and a breast tumor that was surgically removed. Evaluation of the follicular ovarian status by measurement of serum anti-Müllerian (AMH) levels and antral follicle count (AFC) was systematically performed before exogenous FSH administration. FORT was determined by the ratio between the pre-ovulatory follicle count (PFC, 16–20 mm) on the day of oocyte triggering (dOT) × 100/AFC measured just before initiation of the stimulation (d0).
Main results and the role of chance: Overall, women in the letrozole and control groups were comparable in terms of age (34.6 ± 0.8 vs. 33.4 ± 0.4 years, *NS*, respectively), BMI (21.3 ± 0.5 vs. 21.5 ± 0.4 Kg/m^2, *NS*, respectively) and makers of the follicular ovarian status (AFC: 18.7 ± 1.7 vs. 18.5 ± 1.8 follicles, *NS*; AMH: 3.1 ± 0.4 vs. 3.2 ± 0.6 ng/mL, *NS*). After comparable initial doses and total amount of exogenous FSH (2965 ± 134 vs. 2849 ± 167 IU, *NS*, respectively), the number of PFC did not differ between both groups (10.7 ± 0.7 vs. 10.2 ± 0.5 follicles, *NS,* respectively*)*. As a result, FORT index were comparable in patients having received or not letrozole supplementation during COH (33.9 ± 3.4 vs. 37.6 ± 2.7%, *NS,* respectively*)*, as well as the number of oocytes vitrified (9.9 ± 5.6 vs 9.4 ± 7.2 oocytes, *NS*, respectively).
Limitations, reasons for caution: FORT presents inherent limitations. Indeed, FORT calculation assumes that small antral follicles, respond in a coordinated manner to FSH and that only follicles having reached diameters ranging between 16 and 20 mm on dOT effectively responded to FSH. In addition, the sample size may limit the generalization of these findings.
Wider implications of the findings: The present findings indicate that the percentage of antral follicles that successfully respond to FSH administration (FORT) is not modified by letrozole supplementation during COH when compared with conventional stimulation protocols. This is in keeping with the hypothesis that follicle-oocyte quality is not affected by the use of aromatase inhibitors.
Trial registration number: N/A.

P-492 Sphingosine 1-Phosphate does not improve mice follicle culture survival after treatment with cyclophosphamide

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Study question: Does a medium enriched with Sphingosine 1-Phosphate (S1P) improve mice follicle survival in culture after administration of cyclophosphamide (CPA) *in vivo*?
Summary answer: Addition of S1P to the culture medium did not improve follicle survival in culture or follicle growth development parameters.

What is known already: Alkylanting agents including CPA are highly gonadotoxic and treatments that include such drugs induce follicle depletion and infertility. Sphingosine 1-Phosphate (S1P) is an anti-apoptotic agent that promotes cell growth and mitosis, improving not only cell proliferation but also suppressing cell death. S1P has been tested in reproductive experimental settings *in vivo* and it seems effective in improving cell survival, but no previous data on the investigation of S1P in follicle culture of treated mice with CPA are available.
Study design, size, duration: Randomized experiment. Twenty B6CBA/F1 mice, four weeks old, were randomly assigned (n = 5/group) to treatment with CPA (single injection) Group I: 100 mg/Kg, Group II: 75 mg/Kg, Group III: 50 mg/Kg, and Group IV: control. All mice were sacrificed after 72 h and secondary follicles were isolated. The follicles obtained from each treatment condition were thereafter assigned to culture media enriched with S1P (50 nM), or without. The study was approved by the ethics review board for animal experiments.
Participants/materials, setting, methods: After isolation, the secondary follicles were cultured under oil in a microdrop system for 12 days, according to the method described by Cortvrindt et al. (1996). Follicle growth was monitored daily and follicle size and morphology registered.
Main results and the role of chance: The groups of mice treated with CPA presented a 60% reduction in the number of secondary follicles that could be isolated, when compared to the control group ($p < 0.001$). However, the numbers of follicles isolated in the three CPA treated groups were similar and did not differed significantly across the different dose groups.

Follicle survival rates in culture were similar in the three groups treated with CPA, but also when compared those to the control group. Follicle growth and final follicle size were also similar between all the groups. No improvements either in survival rate or in final follicle size were observed in follicles cultured in medium that was enriched with S1P, independently of the treatment condition.
Limitations, reasons for caution: The growth of follicles *in vitro* was monitored only by established morphological characteristics. Additional investigation of molecular features would have provided further information on follicle development *in vitro*.
Wider implications of the findings: Our study shows that mice secondary follicles, previously treated with CPA, can be isolated and cultured. Despite a significant reduction in the number of follicles collected from treated CPA ovaries, *in vitro* follicle growth was similar. Addition of S1P to culture media did not improve growth or follicle survival.
Trial registration number: basic science study

POSTER VIEWING SESSION

PARAMEDICAL - LABORATORY

P-493 The effect of pre-warming the prepared sperm fraction for conventional IVF: a prospective sibling oocyte study

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Study question: This prospective sibling oocyte study evaluated whether pre-warming the prepared sperm fraction at 37°C for 1 h before IVF insemination improves fertilization and embryo development.
Summary answer: Sperm pre-warming for 1 h at 37°C does not improve fertilization and embryo quality compared to sperm kept at room temperature (control).
What is known already: Hyperactivation of spermatozoa, a process needed in order to fertilize the oocyte, is temperature dependent and faster induced at 37°C. However, preservation of capacitated sperm at 37°C during several hours before IVF insemination is discouraged because of the high energy consumption before fertilization can take place. Pre-warming the prepared sperm fraction for 1 h at 37°C could assist the spermatozoon to undergo the hyperactivation process and thereby facilitate fertilization and developmental capacity.
Study design, size, duration: A prospective sibling oocyte study was performed from September 2014 until January 2016, including 93 IVF cycles with at least 4 cumulus-oocyte complexes (COC). Semen was prepared on a 90%/45% density gradient (Spermient, COOK). The prepared sperm fraction was divided in two aliquots. In the control group, the prepared sperm fraction was kept at room temperature until insemination, while sperm was incubated for 1 h at 37°C immediately before insemination in the study group.
Participants/materials, setting, methods: The oocytes (93 cycles) were cultured in 25 µl droplets of fertilization medium (Origio). Either the oocytes of the control group or the oocytes of the study group were first inseminated, based on a computer-generated randomization list. To each droplet, 10000 progressively-motile spermatozoa were added. Inseminated oocytes were incubated overnight (16–20 h) until denudation. Statistical analysis was performed by the Chi square test, a p-value <0.05 was considered significant.
Main results and the role of chance: A total of 1064 sibling oocytes were inseminated, 533 in the control group and 531 in the study group. The fertilization rates in the control group (59.1%) and the study group (59.9%) were comparable ($p = 0.842$). On day 3, there was no difference in the proportion of excellent or the proportion of good-quality embryos between the groups ($p = 0.259$). The same observation was made for the 217 embryos (control group) and 229 embryos (study group) that were further cultured till day 5/6 ($p = 0.126$). Finally, no difference was found in the utilization rates (proportion of embryos transferred or frozen per COC) of the control and the study group (29.5% versus 29.8%, $p = 0.968$).
Limitations, reasons for caution: The overnight incubation of oocytes with sperm might have counteracted the possible effect of 1 h pre-incubation of sperm. The probability of a beneficial effect of a short pre-warming period might be higher when a short 2-h co-incubation of oocytes and sperm is performed.
Wider implications of the findings: With respect to our standard protocol of overnight co-incubation, pre-warming sperm for 1 h at 37°C seems not beneficial, thus sperm will be kept at room temperature before insemination. However, a consecutive similar study with co-incubation of oocytes and sperm during only 2 h can be considered.
Trial registration number: None

P-494 Evaluation of two different types of time-lapse embryo observation system

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Study question: Are there different outcomes between the two different time-lapse embryo observation systems, EmbryoScope and PrimoVision?
Summary answer: Although better outcomes are expected with PrimoVision, having a group culture system, no significant difference was detected in the time-lapse parameters and pregnancy rates.
What is known already: Since time-lapse systems became available, some parameters, such as speed and timing of embryo development were reported to predict good embryos. Group culture systems have been reported to improve embryo development rates and pregnancy rates. Closed incubator systems are considered to produce similar results, as compared to conventional open incubator systems. Although there have been reports comparing closed and open systems, there was previously no reported data which compared the culture dishes used in each system.
Study design, size, duration: Comparison of two different types of time-lapse system (EmbryoScope: a closed incubator with individual culture, PrimoVision: an open incubator with group culture). This was a retrospective cohort study involving 2,372 embryos from 270 women (<40 years old) who underwent blastocyst culture using the two time-lapse systems, and cryopreservation of their all blastocysts between September 2013 and October 2015. Patients were allocated randomly to each time-lapse system. Mouse embryo testing was performed on culture dishes.
Participants/materials, setting, methods: The time from pronuclear membrane break down (PNMBD) to 2–5 cells and 8 cells (tpnb2–tpnb5, tpnb8, from 2 to 3 cells (cc3), from 3 to 4 cells (s2), from 5 to 8 cells (s3) were compared between the two systems. Embryo development rates, pregnancy rates, and abnormal cleavage rates were compared between the two systems. The mouse

embryo test was performed in ES and PV dishes in the same open system incubator.
Main results and the role of chance: Blastocyst development rates in ES and PV were 57.7% (357/619) and 53.4% (339/633) and high quality blastocyst (≧G3BB) development rates in ES and PV were 26.5% (164/619) and 26.9% (170/633). Chemical and clinical pregnancy rates were 66.7% (58/87) and 60.9% (53/87) in ES and 61.4% (35/57) and 50.9% (29/57) in PV. Abnormal cleavage rates, including reverse cleavage and direct cleavage from 1 cell to more than 2 cells and from 2 cells to more than 4 cells were 9.2% (8/87) in ES and 8.8% (5/57) in PV. The parameters tpnb2, tpnb3, tpnb4, tpnb5, tpnb8, cc2, s2 and s3 were 27.0 ± 3.4, 37.6 ± 4.9, 38.7 ± 4.6, 50.9 ± 7.1, 58.9 ± 9.2, 10.5 ± 3.0, 1.2 ± 1.9, 8.0 ± 7.4 h in ES, and 27.5 ± 3.3, 37.4 ± 5.2, 38.2 ± 7.4, 50.7 ± 7.4, 59.7 ± 8.0, 9.6 ± 4.1, 1.2 ± 2.1, 8.7 ± 7.4 h in PV respectively. In all parameters, there was no significant difference between ES and PV. The mouse embryo test showed that the blastocyst development rate was significantly higher in PV dishes (78.1%) than in ES dishes (30.4%) ($p = 0.0011$). No significant difference was detected between the ES dish culture (30.4%) and the control (individual) culture (36.4%), whereas the blastocyst rate was significantly higher in the PV dish culture (78.1%) than in the control (group) culture (31.3%) ($p = 0.00016$).
Limitations, reasons for caution: As only patients who are younger than 40 are enrolled in this time-lapse observation, the results may not be enhanced to assist patients who are older than 40.
Wider implications of the findings: As there was no statistical difference between the two time-lapse systems, the merits in each system might counterbalance each other. A time-lapse system, which employs both a group culture system and a closed incubator system, using a less toxic culture dish, will improve ART outcomes.
Trial registration number: Not applicable.

P-495 Association of follicular fluid zinc concentration with maturation rate and fertilization rate during ovarian stimulation

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Study question: Is zinc concentration in follicular fluid obtained from women during ovarian stimulation involved in human oocyte and embryo development?
Summary answer: Zinc concentration in follicular fluid appears to be involved in human oocyte maturation and fertilization rate.
What is known already: Zinc is an essential trace element during early embryogenesis for the stabilization of DNA conformations in association with DNA repair. It also has an important role in preventing high levels of reactive oxygen species during early embryogenesis.
Study design, size, duration: A retrospective study of *in vitro* fertilization (IVF) during October 2013 to May 2015 on 117 women. Among them, 59 women were treated with GnRH agonist short protocol and 58 women with GnRH antagonist protocol. All IVF cycles were carried out with informed consent at our clinic. The primary outcomes of this study were to analyze the maturation rate of human oocytes and the fertilization rate and their association with intra-follicular concentration of zinc.
Participants/materials, setting, methods: The follicle fluid was obtained from the largest aspirated follicle without any blood contamination. Oocytes were collected from all follicles and fertilized with ICSI. The maturation rate, fertilization rate were evaluated. Zinc concentration of follicle fluid was measured using a metal assay kit. Statistical analyses were performed using *Welch's t-test* and multiple logistic regression analysis. A statistical significance was considered as $P < 0.05$.
Main results and the role of chance: Mean age of combined GnRH agonist and antagonist women was 38.2 years and the maturation rate and fertilization rate were 79.3% and 55.5%, respectively. There was no significant difference in age between GnRH agonist group (37.6 years.) and antagonist group (38.8 years., $P = 0.071$). However, a significant difference was observed between these two treatment groups in maturation rate (75.3 vs. 83.3%, $P = 0.024$) and zinc concentration (50.8 vs. 54.9 μg/dL, $P = 0.011$). Multiple logistic regression analysis indicated that the maturation rates of human oocytes were influenced by the difference of follicle stimulation protocols ($P = 0.003$) and follicular fluid zinc concentration ($P = 0.007$) regardless of the age ($P = 0.44$). The follicular fluid zinc concentration was negatively correlated with maturation rates. In contrast, the fertilization rates were influenced by follicular fluid zinc concentration ($P = 0.01$) regardless of the age ($P = 0.58$) and the difference of follicle stimulation protocols ($P = 0.69$). Our findings suggest that measuring follicular fluid zinc concentration may predict outcome of oocyte maturation and fertilization rate in women during different ovarian stimulation.
Limitations, reasons for caution: At our clinic, we commonly choose GnRH agonist short protocol during follicle stimulation for women with first IVF cycle. This may increase the risk of selection bias. Small sample size in each stimulation group in this retrospective study should be carefully considered to decide final conclusion.
Wider implications of the findings: Different stimulation protocol may be involved in variable zinc concentration of follicle fluid. In ART clinical practice, GnRH antagonist protocol may be recommended to women showing less oocyte maturation with GnRH agonist protocol. Measurement of zinc concentration in follicular fluid may help decide the selection of next ovarian stimulation protocol.
Trial registration number: None

POSTER VIEWING SESSION

PARAMEDICAL - NURSING

P-496 In-vitro fertilization with donor sperm versus social freezing in single women: the importance of family proximity

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Study question: Are motivations and personal characteristics of single women undergoing IVF with donor sperm (IVF) different from those undergoing social freezing (VIT)?
Summary answer: IVF women live closer to their families, and perceive to have a stronger family support than VIT women do.
What is known already: The decision to have a child might be postponed by the lack of a partner, and social freezing can afford single women more time to find a suitable companion to reach the desired family structure. Alternatively, some women decide not to postpone motherhood and to have a child on their own thorough IVF with donor sperm. Both procedures can be seen as solutions to permanent involuntary childlessness in single women, but the motivations of women undergoing one treatment or the other, and their characteristics, have never been investigated.
Study design, size, duration: This cross-sectional study includes 250 single women from 4 European countries (Spain, France, Italy, and United Kingdom) who underwent either IVF with donor sperm (192) or social freezing (58) between January and December 2015 in a large fertility center.
Participants/materials, setting, methods: Study participants were heterosexual single women; an anonymous electronic survey was sent after the women started the elected treatment. The survey consisted of 18 close-ended or multiple choice questions. The estimated time to fill in the survey was 5 min. The response rate was 49.8%. Predictors to be part of the IVF or the VIT group were analyzed following a logistic regression model. Patients' characteristics were also analyzed by logistic regression.
Main results and the role of chance: Demographic characteristics between IVF and VIT women were similar (mean age 38.5), and 3/4 reported having been single for more than one year. However, women in the IVF group lived closer to their original families than those in the VIT group, close enough to visit with family once a week (OR 3.9, 95% CI 1.9, 8.1, $p < 0.001$), and received a higher emotional and/or financial support from them (OR 2.5, 95% CI 1.1, 5.6, $p = 0.030$). We observed slight differences in education and occupation: while most participants in both groups are highly educated and have a job, more VIT hold an advanced university degree -master or doctorate- (68 vs. 33.2%, $p < 0.001$), while more IVF are employees rather than for instance self-employed (80.5 vs. 65.5%, $p = 0.017$). Regarding motivations, the most common reported reason for not having fulfilled the motherhood desire was a lack of partner (72.5% of IVF and 62.1% of VIT), as expected. However, more VIT

reported a late desire to have children (15.5% VIT vs. 6.3% IVF, $p = 0.029$), and considered having a partner more important than having a child (12.1% of vs. 0.0% IVF; $p < 0.001$). Finally, 100% of VIT knew about the possibility of IVF, while 62% of IVF knew about VIT.

Limitations, reasons for caution: Participants in the study were women who attended the center for undergoing IVF or VIT. No information was collected from women who attended an initial visit but did not follow through with treatment.

Wider implications of the findings: Our results underscore the relevance of family ties in the decision to undergo IVF as single women. Moreover, IVF women might not be fully informed about social freezing as an option to postpone motherhood. Health professionals should be aware of these differences when counseling single women on fertility choices.

Trial registration number: NA.

P-497 The effectiveness of brief mindfulness-based stress reduction program on stress, mindful awareness attention, sleep quality in infertile women: a quasi-experimental study

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Study question: Is the 4-week Brief Mindfulness-based Stress Reduction (B-MBSR) Program effective on reducing stress and increasing mindful awareness attention and sleep quality for infertile women?

Summary answer: Four-week B-MBSR Program can decrease stress and increase mindful awareness attention and sleep quality in infertile women.

What is known already: The infertile women experienced physiological, psychological, and social stress, especially during the infertility treatment period. Mindfulness-based stress reduction program was used widely for patients with chronic disease; however, the effects for infertile women are not clear yet.

Study design, size, duration: Quasi-experimental design study was used in the study. Seventy-three subjects were included, 40 for experimental group and 33 for control group respectively. The experimental group received a 2-h B-MBSR program in every week for four weeks, and conducted daily self-practice for half hour every day. Control group kept their usual daily life style. Evaluation measurement was assessed for both experimental group and control group at baseline (pre-test), 4th week (post-test), and 8th week later (follow-up).

Participants/materials, setting, methods: Infertile women who had been treated routinely at infertility clinic, aged 2–45, understand mandarin, without hearing impairment, and willing to perform home-practice, were recruited. Purposive sampling was used to assign 73 participants into experimental and control group. The study was conducted at outpatient clinics located in northern Taiwan. Perceived Stress Scale (PSS), Mindful Awareness Attention Scale (MAAS), and Pittsburgh Sleep Quality Inventory (PSQI) questionnaires were used to assess effects of the BMSR program.

Main results and the role of chance: After the B-MBSR program, participants in the experimental group showed significantly decreased in the PSS ($B = -4.68$, $p < 0.001$) at the 4th week post-test and continuous decreased ($B = -6.14$, $p < 0.001$) at the 8th week follow-up; the MAAS significantly increased at the 4th week post-test ($B = 4.23$, $p = 0.05$) and continuous increased ($B = 7.43$, $p = 0.001$) at the 8th week follow-up; the PSQI significantly decreased at the 4th week post-test ($B = -1.22$, $p = 0–038$) and continuous decreased ($B = -2.74$, $p < 0.001$) at the 8th week follow-up. However, control group did not change significantly.

Limitations, reasons for caution: Because of clinical setting and ethical issues, randomization cannot be applied in the current study. Physiological indicators of effects, such as stress and sleep quality did not included in the study, that are recommended for next-step study in the future.

Wider implications of the findings: The B-MBSR program was confirmed to be an ideal strategic intervention to reduce stress for infertile women, and feasibility and cost-effectiveness are also setup in the study. Therefore, the B-MBSR can be applied to clinical setting for infertile women.

Trial registration number: N/A

P-498 Is hysterosalpingography really painful?

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Study question: What information can be provided to hysterosalpingography patients to assist in the reduction of expected and/or actual pain levels?

Summary answer: The provision of accurate information may play a part in decreasing the pain levels of hysterosalpingography patients.

What is known already: Patients can easily obtain information from the internet and media nowadays and many of them have preconceptions about hysterosalpingography as very painful. It is known that negative perceptions regarding pain can often increase the level of pain. Thus, this study aimed to analyze the expected pain levels and postprocedure pain levels of patients who underwent hysterosalpingography.

Study design, size, duration: A questionnaire survey was conducted from April 2013 to May 2013, involving 265 patients who underwent hysterosalpingography and agreed to fill out a questionnaire. Patients were given the questionnaire immediately after they underwent the hysterosalpingography.

Participants/materials, setting, methods: The pain-level questionnaire was based on the Numerical Rating Scale (NRS), ranging from level 1 to 10. The pain was also evaluated within three categories: lower, the same or higher than expected. It was further analyzed based on the patency of oviducts. The questionnaire was handed out to patients after obtaining informed consent, including clauses regarding no invasion of privacy and a guarantee against any disadvantages if they did not wish to respond.

Main results and the role of chance: The response rate of the questionnaire was 83.3%. The rates of patients who responded that the pain was lower than expected, the same as expected, or higher than expected were 64.2% (170/265), 20.4% (54/265), and 15.4% (41/265), respectively. The average score regarding expected pain was 6.2 (±2.3) which was significantly higher than the postprocedure pain score of 4.2 (±2.6) ($p < 0.001$).

Among patients who were not found to have oviductal patency, the rates of patients who answered that the pain was lower than expected, the same as expected, or higher than expected were 47.9% (23/48), 20.8% (10/48), and 31.3%(15/48) respectively, whereas they were 67.9% (146/215), 14.4% (31/215), and 36.0% (38/215) respectively in patients who were found to have oviductal patency. A significant difference in pain levels was detected between patients with or without oviductal patency ($p = 0.029$).

Limitations, reasons for caution: As this study aimed to detect pain levels caused by misinformation and/or false preconceptions, further research is needed to confirm if patients' anxiety levels can be reduced by the provision of information based on our findings.

Wider implications of the findings: As nearly a half of patients without oviductal patency reported the pain to be lower than expected, it may be possible to reduce anxiety levels by providing precise information, especially to patients who are reluctant to undergo hysterosalpingography, due to their excessive anxiety about pain.

Trial registration number: Not applicable.

P-499 "Your Fertility" – evaluation of a health promotion program to improve awareness of factors that affect fertility

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Study question: Can a health promotion program improve awareness of potentially modifiable factors that affect fertility?

Summary answer: Data collected over five years indicate that the Your Fertility' program meets a need for targeted, evidence-based, accessible fertility-related information.

What is known already: Knowledge among people of reproductive age about the factors that influence fertility and reproductive outcomes, including ART outcomes, is generally low. The most important factors affecting fertility are parental age, smoking, obesity, and mistiming of intercourse. It

is estimated that 50% of subfertility could be avoided if these risk factors were eliminated. Initiatives to improve awareness about the limitations of the reproductive life span and the impact of health behaviours, including how fertility can be optimised by timing intercourse to coincide with the fertile window, are needed.

Study design, size, duration: Your Fertility' is a health promotion program funded by the Australian and Victorian governments which aims to improve awareness among people of reproductive age and primary health care providers about the potentially modifiable factors that affect fertility and reproductive outcomes. To identify knowledge gaps and guide program development and implementation, qualitative and quantitative formative community research was conducted at program initiation in 2011. Program reach was evaluated over 5 years.

Participants/materials, setting, methods: Collation and analysis of data collected including website activity, social media engagement, health care professionals' uptake of educational activities, and traditional media coverage.

Main results and the role of chance: The formative research revealed considerable knowledge gaps about factors that affect fertility and reproductive outcomes. Factors including age, weight, smoking, alcohol consumption and timing of intercourse were targeted in the development of information materials and informed the dissemination strategy. To optimise reach, information materials and resources were disseminated through a dedicated website, social marketing, media, public relations, and health professional organisations. The Your Fertility' website is the first non-commercial website listed in a Google search. Since its launch in 2011 the annual number of visits to the website has grown exponentially and reached more than 2.6 million in 2015. Over the five years more than 68 million people have been reached through social media campaigns and the most commonly accessed resource is a guide to when in the menstrual cycle a woman is most likely to conceive (almost 5 million views). More than 4,000 health professionals have undertaken training modules on fertility awareness and pre-conception health which are available on the website. Based on published audience figures the number of people who were exposed to information about Your Fertility' through traditional media reached over 86 million.

Limitations, reasons for caution: It is not possible to know if those who access information through the program change their behaviour and thereby improve their fertility. It is also acknowledged that factors beyond personal control affect fertility, such as medical conditions and not having a partner, and these may not be amenable to change.

Wider implications of the findings: The knowledge gaps identified in formative community research and the extensive reach and use of the resources offered by the Your Fertility' program confirm that it meets a need for public and health professional education about the impact of age and health behaviours on fertility and reproductive outcomes.

Trial registration number: Not applicable.

P-500 "The IVF nurse" in infertility care in Italy: a pilot study

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Study question: Nurse can represent an additional resource both for the doctor and for the couple in infertility work-up.

Summary answer: The relationship with nurse permit explanation of doubts, improve the terapeutic communication "couple – doctor – nurse", determining an appropriate and effective pathway of care.

What is known already: Nurse autonomy is a recent achievement in Italy. The idea that exist or there may be a "nurse consultation" scare the insiders and is perceveid as an illusion. In this sense, in Italy, a clear and definite "nurse moment" is not provided in infertility. As a consequence, there are only daily limited time sessions, basically telematic, regarding injection administration, but not helpful for establishing a deep communication with the couple.

Study design, size, duration: This observational cohort study of the duration of six months from January to June 2015 was conducted on 150 couples applied to "Futura Diagnostica Medica PMA" in Florence, for infertility problems.

Participants/materials, setting, methods: The 150 couples was sorted out into 2 groups: Group A: 100 couples, only standard medical consultation.

Group B: 50 couples, nurse consultation following the standard medical consultation. Couples in group B have been enrolled according to infertility nurse availability.

The nurse consultation lasted 30/40 min with slide presentation on infertility techniques and medicine administration; at the end a semi-structured questionnaire on quality's perceived by the couple was administered.

Main results and the role of chance: Assessment survey on couples of group B has demonstrated:

Empathic communication: 90%
Evidence of displayed materials: 95%
Doubts persistence: 30%
Request for additional written material: 25%.

Furthermore, the sample (A + B) was evaluated during the following IVF treatment through a retrospective analysis of clinical record regarding the following items:

Errors during therapy administration: A (2%), B (0%)

Proper competence towards medical procedures (oocyte collection, embryo transfer, testicular sperm retrieval): A (95%), B (99%)

Following telematic contact with medical staff >**2**: A (85%), B (42%)

Following telematic contact with nurse staff >**2**: A (73%), B (55%)

Limitations, reasons for caution: The study has two limitations, the modality of recruitment of patients and the limited size of the sample examined.

Wider implications of the findings: Our study shows that "nurse consultation", in infertility couples, is appreciated by the patients, reduces the rate of errors during treatment and allows optimization of human resources in the IVF centre, combining the different skills of the staff.

Trial registration number: None.

P-501 Sustainable development of nursing internet platforms in the field of reproductive medicine

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Study question: To explore whether the nurse–patient communicating online platform can improve efficiency and quality of nursing service in the field of reproductive medicine.

Summary answer: Online platforms can improve nursing efficiency and quality, and provides a new way for sustainable development of nursing system in the field of reproductive medicine.

What is known already: Network technology has been developed to an unparalleled extent in 21st century, and also healthcare industry is stepping in an informatization age. Internet platform is undoubtedly a favorable way for communication between hospital and patients. It has become a new concern for special nursing personnel in reproductive medicine area that in which way internetwork can be utilized in the new environment to carry out patient health education, promote nurse–patient communication and provide convenient, simple and effective nursing care for patients.

Study design, size, duration: A questionnaire survey was conducted with 517 follow-up patients in our reproductive medical center from May to June of 2015, in order to investigate the utilization of various internet service platforms and patients' satisfaction, aiming at providing reference for the exploration of multiform health education and other special nursing models.

Participants/materials, setting, methods: Internet service platforms were utilized since 2010 in our reproductive center, including department website, QQ (an online-chat software) groups for doctor–patient communication and public WeChat platform (a mobile APP). Major content of the questionnaire composed of 10 choice questions, including awareness and basic usage of various informationization service platforms; which platform was selected separately for appointment register as well as acquisition of assisted reproduction knowledge, satisfaction with the use of various platforms, etc.

Main results and the role of chance: The average age of participating patients was 31.4 years; the average infertility duration was 5.0 years. The survey findings revealed that patients had high awareness rate and usage rate of network service platforms already built by the reproductive center. The WeChat platform of the reproductive center held the highest awareness rate (88.6%) followed by the website of the reproductive center (84.1%). The WeChat platform had the highest usage rate, being 77.4%. Hospital visit appointment with new informationization service platforms was 49.4%, which had exceeded traditional appointment mode. A majority (62.5%) of patients obtained relevant information through the WeChat platform. This figure had far exceeded traditional method of newspaper and magazines (3.5%). Most patients thought WeChat service platform was the most convenient one to use (60.3%) and also the most useful

one for information acquisition (62.4%). In this survey, most patients expressed their satisfaction with various network service platforms of the center. The overall satisfaction rate was over 70%.
Limitations, reasons for caution: Differences in region, education degree and economy situation can result in differences in ability of using information technology, which therefore may give rise to bias of our result.
Wider implications of the findings: In China, network service platforms of the reproductive center are still in an exploratory stage. How to further use of network service platforms and encourage patients actively utilize network platforms is the focus of continuous development. Nursing in reproductive medicine should fully use network resources to realize high-quality nursing.
Trial registration number: Empty.

P-502 Poor semen quality and effect on masculinity, and on couple and family formation

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Study question: How does poor semen quality affect the masculinity, the couple and their thoughts of family formation?
Summary answer: The low semen quality was threatening the men's masculinity. The participants focused on their primary plan to conceive and have their own biologically children.
What is known already: Infertility concerns both the woman and the man. There is a limited knowledge on how male factor infertility affects the couple in fertility treatment. For men who perceive fatherhood as an important part of their masculinity, male infertility can have significant negative effect on their sense of masculinity. Fertility and masculinity seem to be closely related and to play an important role in the male identity.
Study design, size, duration: Longitudinal, semi-structured qualitative interview study including ten men with very poor semen quality undergoing ICSI-treatment. Participants were interviewed before and after their first ICSI-treatment attempt. The data collection took place between November 2014 and May 2015.
Participants/materials, setting, methods: The participants were assigned to fertility treatment at the Fertility Clinic, Hvidovre Hospital, Copenhagen, Denmark. A total of 15 men were contacted out of which five men declined participation. Ten men were interviewed before their first ICSI treatment attempt, and five men participated in the follow-up interview. The interviews were audiotaped and transcribed in full. Data were analyzed using qualitative content analysis following Graneheim and Lundman.
Main results and the role of chance: The following main themes were found: "Masculinity," "The couple as a patient," and "Different pathways for family formation." The men felt that they were not able to do what a man was supposed to do, and they felt like less of a man. They showed their masculinity by being the protecting and strong partner in their relationship. The men wanted to be addressed individually and not just as a pendant to their partner. More couples had had conflicts and discussions because the women in general wanted to talk more about infertility than the men did. However, they still had many thoughts concerning unsuccessful treatment. The men focused on the goal to have their own biological child. They wanted to push a decision of adoption or donor a side. If it later on should turn out that they would have to use donor, adoption or live without children, then it was a different chapter; a chapter they preferred to postpone. The men wished to focus on their current situation.
Limitations, reasons for caution: A limitation of the present study was the risk of selection bias. In the follow-up interview only five out of the ten men agreed to participate, mainly because it was to stressful for them to be in treatment.
Wider implications of the findings: Fertility staff should create a space and give their patients an opportunity to verbalize their concerns and questions related to male infertility and the different challenges the couple will face in the treatment. Patients need the staff to offer information about how their poor sperm quality could affect their lives.
Trial registration number: None.

POSTER VIEWING SESSION

PSYCHOLOGY AND COUNSELLING

P-503 Evaluation of the quality of life (QoL) of infertile patients in the public health sector in Chile

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Study question: What is the infertility-specific QoL as perceived by patients in the Chilean public health system? Does it fluctuate according to different bio-demographic variables?
Summary answer: QoL for patients with primary infertility was significantly lower. Couples in rural areas reported better QoL regarding personal issues, but worse satisfaction with medical services.
What is known already: Infertility and its treatment are known to produce significant emotional distress and a reduction in personal well-being. The scientific literature provides substantial evidence of these negative effects. The Fertility Quality of Life Questionnaire (FertiQoL et al., 2011) was developed to measure quality of life that is specifically associated to infertility in a single international instrument and has been applied mostly in English speaking/European populations. Little is known about the quality of life of infertile patients in developing countries, where cultural norms on reproduction and family-building are presumably more traditional and access to reproductive treatment more limited.
Study design, size, duration: Descriptive, cross-sectional study conducted at the Instituto de Investigación Materno Infantil, Hospital Clínico San Borja-Arriarán, Universidad de Chile, from May through November 2015. This institution is a government-appointed national center providing first-line and assisted reproduction treatments of infertility covered by public funding. In Chile 72% of the population is under care of public health services. Participation in the study was voluntary and anonymous.
Participants/materials, setting, methods: The complete FertiQoL (in certified Spanish version) was applied to 132 patients (40 men and 92 women) upon enrollment in the *in vitro* fertilization program and prior to initiating treatment. Scoring scales range from 0 to 100, representing an increasing quality of life. In addition, bio-demographic data were collected. Subgroups were compared using independent *t*-tests and one-way analysis of variance where appropriate.
Main results and the role of chance: No significant differences were found in FertiQoL scores as a function of age, gender (though men overall scored higher), educational level, and duration of infertility. However, significant differences were observed as a function of parity and cause of infertility: patients with secondary infertility scored higher than patients with no previous children (overall mean scores 73.9 and 65.9, $p < 0.001$) and patients with combined aetiology scored significantly lower on the CORE domain (emotional and social issues) than those with other aetiologies (mean scores 63.0 and 74.4, $p < 0.01$). In addition, patients from rural areas scored significantly higher than patients from urban areas (75.6 and 68.1, $p < 0.01$) in the CORE subscale, but significantly lower (57.8 and 64.8, $p < 0.05$) on the TREATMENT subscale (satisfaction with medical issues). All groups uniformly assigned the highest score to the RELATIONAL domain (marital satisfaction). The lowest score was systematically assigned to the ENVIRONMENT domain (quality of clinical services). The correlations between domains and total scores of FertiQoL were positive and statistically significant, with the exception of ENVIRONMENT which did not correlate significantly.
Limitations, reasons for caution: The relatively moderate size of the sample may limit the generalization of results (small aetiology categories). The present data are part of an ongoing, larger scale study to be completed during 2016. These data provide no information about infertile patients' perception of quality of life in private clinics.
Wider implications of the findings: This study provides useful cross-cultural information about patients' experience of infertility and its impact on quality of life. It contributes to the validation of FertiQoL in a Latin American

Spanish-speaking country. It offers specific evidence on: men's experience, infertility aetiology, existence of previous children, and is based on clinical recruitment.
Trial registration number: N/A.

P-504 Freezing their fantasies? Linking what we know of "circumstantial (social) childlessness" with an increasing demand for social egg freezing

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Study question: How do women articulate their experience of loss, grieving and the impact of "circumstantial childlessness" in their daily lives?
Summary answer: "Circumstantially childless" women had vivid fantasies of themselves as mothers and the child(ren) they anticipated having, and these had significant material effects in their lives.
What is known already: The incidence of unintentional childlessness in women who have "left it too late," is rising markedly, but women's psychosocial experience of it is little researched and is not well understood. There are no known previous studies of the sense of loss these women experience, what is understood to be "lost," or the grieving and adaptations they make to the likelihood that their fantasies of motherhood and a child may not be embodied. The implications for the take-up of oocyte cryopreservation is not known.
Study design, size, duration: A qualitative psychosocial study carried out over 3 years across four sites in New Zealand. 28 "circumstantially childless" women aged between 32 and 52 were interviewed individually, and 13 of those women also took part in a semi structured group interview. The study was designed in such a way that a grief response was not presumed.
Participants/materials, setting, methods: Participants were interviewed in a variety of settings. Semi structured individual interviews, group interviews, and an innovative participant-produced drawing exercise, used as part of the group interview, were used to collect data. Narrative, thematic and visual analytical methods were employed.
Main results and the role of chance: The psychosocial methods employed were very effective at collecting data rich in affect and complexity. Central themes that emerged were loss, isolation, a sense of being marginalised, and an ongoing disenfranchised grief that pervaded their daily lives. An unexpected result was the extent to which these circumstantially childless women had often very vivid fantasies of the mother they see themselves becoming, and the child(ren) they want(ed) to have. The loss they grieved was the inability to embody these fantasies. Women had named their (hoped-for) children, bought clothes and toys for them, and planned their major aspects of their lives around their dreams of becoming a mother. The intensity and material effect of these fantasies on their daily lives has important implications for family creation, the reproductive choices circumstantially childless women make, for their take-up of technologies such as IVF or oocyte cryopreservation, for women's wellbeing, and for counselling in clinics. A further study is now in planning to investigate how women conceptualise their frozen eggs; is this a matter of "freezing time," as it is currently understood, or may these frozen oocytes be invested with the fantasies of a maternity and a child?
Limitations, reasons for caution: This was a small study, whose aim was to generate rich qualitative data. The results are not able to be generalised across a wider population.
Wider implications of the findings: The study has implications for the growing popularity and promotion of oocyte cryopreservation. How might "circumstantially (socially) childlessness" women who use (or plan to use) oocyte cryopreservation conceptualise their "frozen eggs" and what are the implications for family creation, storage and disposal of "unwanted" eggs, and women's wellbeing?
Trial registration number: Not applicable.

P-505 Attitudes towards "social egg freezing" from a socio-cultural perspective

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Study question: Goal was to investigate the relationship between fertility knowledge and attitudes towards "social egg freezing" as an impact of different socio-cultural backgrounds ("milieus") in Germany.
Summary answer: There are distinct differences between attitudes toward "social egg freezing" and socio-cultural backgrounds, age cohorts, family status, education, fertility problems – but not fertility knowledge.
What is known already: The tendency to delayed parenthood is still on and driven by the availment of (early) reproductive techniques for planning parenthood in the future. The so-called "social egg freezing" stands for the cryopreservation of a woman's oocytes for non-medical purposes. Since "social egg freezing" is a relatively new and originally for cancer patient designed reproductive procedure, it stirs up many ethical, political and social debates. Until now, there are only few studies investigating social–psychological aspects and attitudes of "social egg freezing." Studies regarding fertility awareness and fertility knowledge as displayed by different socio-cultural backgrounds are missing.
Study design, size, duration: A quantitative online study was devised, approved by the Ethics Committee of the Heidelberg Medical Faculty. The study took place from April to June 2015 with $N = 643$ participants.
Participants/materials, setting, methods: Participants completed a questionnaire with items regarding fertility-specific knowledge and attitudes toward social egg freezing, partly derived from an already tested questionnaire in Belgium (Stoop et al., 2011). Furthermore, items indicating specific milieus from the German DELTA-Institute for Social and Ecological Research as various sociodemographic questions, e.g., family status or fertility problems, have been raised. Data were analyzed using correlation, regression, independent *t*-test analysis and ANOVA.
Main results and the role of chance: In total, $N = 553$ woman (Ø age: 34.2) and $N = 90$ men (Ø age: 37.8) participated in the study. Fertility problems were reported by 34.3% women and 12.5% men; 38.5% women and 33.3% men already had children. Of all participants, 69% were university graduates. The Delta milieus consist of two axes: social stratum (e.g., upper class) and basic orientation (common traditions, self-realization, self-management). The milieus "Postmaterielle," "Performer," and "Expeditive" (middle to upper class, mainly self-realization and self-management) were disproportionate high, while the delta milieus "Traditional," "Disadvantaged" and "Hedonists" (lower to middle class, mainly common traditions and self-realization) were disproportionately low distributed. There were distinct differences between attitudes toward "social egg freezing" and various age cohorts, family status, educational achievement, fertility problems and socio-cultural backgrounds. In the context of fertility specific knowledge, only 15% of all participants were able to answer correctly more than half of the questions. However, there was no correlation between fertility specific knowledge and distinct socio-cultural backgrounds or attitudes towards "social egg freezing."
Limitations, reasons for caution: Generalization of findings is limited due to the nature of online surveys, above-average existence of fertility problems and high educational background of participants as unequal participation from people of different socio-cultural backgrounds.
Wider implications of the findings: The investigated differences subject to socio-cultural backgrounds suggest a concerted research to acquire a better comprehension of notions and expectations in the field of desire of children in the German society to develop focused educational interventions. Furthermore, the little knowledge of reproductive methods indicates information deficits needful to be dissolved.
Trial registration number: No clinical trial.

P-506 Infertility related communication and coping strategies among women affected by fertility problems in Sweden

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Study question: The aim was to describe infertility related communication and coping strategies among women affected by fertility problems in Sweden.

Summary answer: Thirty percent of participants had difficulties discussing infertility related subjects with their husband or with close other people, and they avoided socializing with pregnant women.
What is known already: Involuntary childlessness is a reality for many reproductive aged couples and can be psychologically stressful for many of them. The inability to have children affects couples worldwide and causes psychological and emotional distress on them. Both involuntary childlessness and infertility treatment places pressure on those involved. Difficulty in marital communication and high use of active-avoidance coping are significant predictors of high fertility problem stress.
Study design, size, duration: A case control study design involving 199 Swedish women (mean age 36.3). The participants received a letter regarding information of the study including the questionnaire and a postage-paid reply envelope. Data was collected during January to May 2012. Approval from the Ethical Review Board, Stockholm (EPN 2006/1025-31; EPN 2009/5:9) was obtained.
Participants/materials, setting, methods: The population consists of women with history of primary infertility, minimum of 1 year, which sought to a fertility clinic voluntarily or was referred from other clinics. A self-administered semi structured questionnaire inspired by (COMPI) was used to collect the data. Descriptive statistical methods were used to analyse the data.
Main results and the role of chance: Response rate was 66%. Seven percent of the population did not discuss the inability to have a child, and 9% did not discuss about reasons why they were childless at all with their spouse. Seventy-five percent discussed infertility related subjects only to close other people and 20% did not discuss about the results of examinations and tests with people outside of the family. Thirty-five percent did not ask other childless individuals, friends or relatives for advice and 15% were not able to discuss about how tests and treatments affect them emotionally. Twelve percent reported that they leave the room when the subject children or pregnancies are discussed.
Limitations, reasons for caution: Participants were recruited from one infertility clinic from great Stockholm area. The population consists of 199 participants and 84% of them had postgraduate education.
Wider implications of the findings: By highlighting possible problems with infertility relating communication, physicians, midwifes and nurses at fertility clinics may become aware of this problem and therefore dare to address the question. This knowledge may ensure high quality of care, improve patient wellbeing and possibly higher treatment compliance.
Trial registration number: –

P-507 Oocyte vitrification to postpone maternity: a good medical option that is at present incompatible with social and economic conditions in Spain

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Study question: The objective of this study was to ascertain whether or not fertile women attending a gynaecologist are aware that oocyte vitrification exists and would consider it personally as an option to delay pregnancy.
Summary answer: Young women would not choose to freeze their oocytes. Older women would but are too old to benefit from vitrification. Better patient advice is needed.
What is known already: When maternity is delayed the possibility of falling pregnant reduces drastically and there is an exponential increase in both the possibility of conceiving a chromosomally abnormal fetus and of needing egg donation. Until now freezing oocytes by the slow cooling method gave a low chance of oocyte survival at defrosting and of clinical pregnancy. With the development of vitrification the clinical prognosis for vitrified oocytes has become comparable to that of fresh oocytes.
Study design, size, duration: 200 patients attending a routine annual gynaecological check-up between January and April 2015 were asked to complete a survey. They were of the right age to be considered potential candidates for oocyte vitrification due to social reasons.
Participants/materials, setting, methods: Of the 200 women surveyed, 182 completed the questionnaire correctly. The age ranges were: 6.1% (18–24 years), 21.5% (25–29 years), 37.5% (30–34 years), 33.7% (35–39 years), and 1.1% (40–44 years). They were asked if they wanted to become mothers and regarding social and financial issues that could affect that decision. Although they wanted some in the future, 63.5% did not have children.
Main results and the role of chance: 68% had heard of oocyte vitrification, 16% indicated they wanted to do this (independently of whether or not they already knew of the technique) but only 22.1% said they would be willing to pay for it. 44% said they would freeze eggs between the ages of 35 and 39. The main reasons given for not having children were based on the current difficulties in finding a stable job, and the little financial help received with maternity care. Finally they also expressed difficulty in finding a stable partner.
Limitations, reasons for caution: No limitations.
Wider implications of the findings: An important discrepancy exists between medical advances and society's response: the younger women surveyed would not freeze their eggs, women who would consider it were older, and few would proceed if there were additional costs. We need more effective ways of informing women who would benefit most from this technique.
Trial registration number: –

P-508 Hope and psychological symptoms in infertile couples undergoing assisted reproduction treatment

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Study question: How are hope, depression, anxiety and stress levels among three groups of infertile couples undergoing infertility treatment?
Summary answer: The level of hopelessness was higher in infertile wives than their husbands, while it was not significantly different among females or males.
What is known already: When infertility treatment takes a long time, or failure occurs, infertile patients more likely face hopelessness. The loss of hope to have a child is important because hope is one of the main psychological needs. Previous studies investigated psychological moods in infertile patients and showed that infertile women experience higher psychiatric disorders, especially anxiety, depression, stress, than their partners. Currently, there was no research to quantitatively examine the utility of Snyder's theory of hope in patients suffering from infertility.
Study design, size, duration: A cross-sectional survey was conducted on infertile couples referred to the Royan institute, a referral infertility clinic in Tehran, the capital of Iran, between 2013 and 2014.
Participants/materials, setting, methods: Participants consisted of three groups of infertile couples: 60 couples candidate for oocyte donation, 60 couples candidate for embryo donation, and 60 couples of normal infertile. The mean age was 32.94 ± 4.74 years in men, and 29.39 ± 5.09 years in women. Participants completed three questionnaires: the demographic questionnaire, the Adult Trait Hope Scale by Snyder, and Depression Anxiety Stress Scale (DASS) by Antony et al.
Main results and the role of chance: The mean score of hope was significantly higher in husbands than wives in the normal infertile group ($p = 0.046$). There was no significant difference in male patients ($p < 0.148$), as well as in female patients among three groups studied ($p < 0.735$). No significant difference was seen in the mean score of agency between wives and husbands in each group. In all groups, no significant difference was also observed in male and female patients. In the normal infertile group, the mean score of pathway was significantly higher in husbands ($p = 0.032$), and there was no significant difference in male and female patients among three groups. The distribution of depression was significantly different in male subjects among all groups ($p = 0.01$). The frequency of anxiety (normal, slight, medium, severe, and very severe) was significantly different in female subjects ($p = 0.028$). In the normal infertile group, the distribution of anxiety was significantly different between wives and husbands ($p = 0.006$). The frequency of stress (normal, slight, medium, severe, and very severe) was significantly different in male subjects ($p = 0.048$). In the embryo donation group, stress was significantly different between wives and husbands ($p = 0.002$). In the normal infertile group, stress was significantly different between wives and husbands ($p = 0.05$).
Limitations, reasons for caution: Because this study was cross-sectional, no conclusions on causality were inferred. We relied on patients coming to only one center, which is a referral clinic for infertility. This limits the generalize ability of the findings.

Wider implications of the findings: This was the first study to examine Snyder's construct of hope in special samples of infertile couples, the results suggest that hope may be important in reducing psychological symptoms in infertile couples. It is suggested to hold psychological counseling sessions (hope therapy) to all patients during assisted reproduction treatment.
Trial registration number: None.

P-509 Social oocyte cryopreservation: a portrayal of Brazilian women

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Study question: Would childless women at reproductive age consider oocyte cryopreservation to postpone childbearing? What are their reasons for not doing so now?
Summary answer: Most participants consider safeguarding their reproductive potential. The high cost of the procedure is the main obstacle preventing an oocyte cryopreservation cycle.
What is known already: Recently, the Brazilian Ministry of Health announced that Brazilian women are having fewer children and much later in life. This is possibly due to recent media coverage about age-related infertility; women have gained substantial knowledge about the speed of fertility decline. Women who are aware of the limited female reproductive lifespan have the power to determine and plan their reproductive future. As a consequence, oocyte cryopreservation can now offer women some protection against the decline in fertility associated with aging. However, until now, no study has examined the motivations of Brazilian women with regards to social oocyte cryopreservation.
Study design, size, duration: Women at reproductive age who were randomly selected from the general population using different e-mail lists were contacted by e-mail and invited to participate in the study by completing an online web survey regarding social oocyte cryopreservation. Participants who answered yes when asked if they have had children were automatically excluded from the study. The survey was performed from May 2015 to November 2015. There was no incentive for completing the survey.
Participants/materials, setting, methods: Questionnaires from 444 childless women ($\mu = 33.5$ year; 17–44) were included. Incomplete questionnaires were eliminated. Sixty-six percent (295/444) of women were university educated. Data management and analysis were conducted using StatsDirect statistical software. A multivariate logistic regression analysis was performed to identify the factors affecting the women's decision regarding oocyte cryopreservation.
Main results and the role of chance: Although most of our responders have a partner (86.9%; 386/444) and have already planned the pregnancy of their first child (69.6%; 309/444), 85.4% (379/444) considered the potential of social oocyte freezing to improve their chances of giving birth later in life. Those that had already planned pregnancy were 2 times more likely to freeze their oocytes (OR: 1.95; 95% CI 1.06–3.60; $p = 0.03$). An increase in wage level increased the odds of oocyte cryopreservation by 24%. The most important barrier for not undergoing oocyte cryopreservation was the cost. The women who indicated that they could not currently undergo the procedure now due to cost had a 2-fold (OR: 2.1; 95% CI 1.07–4.04; $p = 0.03$) greater intension to cryopreserve their oocytes than women who thought that they would not need to delay pregnancy. The study population had negative views about receiving a sperm donation even if that were the only alternative for them to become mothers (63% not accept; 280/444).
Limitations, reasons for caution: Our study population is characterized by a higher educational level and a reasonable standard of living.
Wider implications of the findings: Brazilian women who think that they are not ready to have a family are discovering the option to cryopreserve their oocytes. Making the procedure more accessible could provide more women with the opportunity to make proactive decisions about the future of their fertility.
Trial registration number: Not applicable.

P-510 The association between the psychological assessment scores and stress biomarker levels on the clinical outcomes of women undergoing ART treatment

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Study question: Are psychological assessment scores and salivary alpha amylase (sAA) levels at the time of embryo transfer (ET) procedure associated with the clinical pregnancy rate of women undergoing ART treatment cycles?
Summary answer: sAA level and psychological questionnaires were not associated with pregnancy rate. sAA levels were significantly correlated with age, Fertility Problem Inventory (FPI) and Perceived Stress Scale (PSS).
What is known already: Previous studies have shown that increased levels of the stress biomarker (sAA) are associated with a longer time-to-pregnancy and infertility in unselected women. However, although there has been data to support that psychological stress may influence ART outcomes, sAA levels have never been examined before within the ART context.
Study design, size, duration: Prospective observational study of 54 consecutive women undergoing IVF/ICSI cycles between September 2015 and January 2016.
Participants/materials, setting, methods: Sample for sAA levels were collected using dedicated Salivettes immediately before and after ET. Women also completed questionnaires on the day of ET, aimed at assessing (i) fertility stress/concerns (FPI), (ii) psychological stress [visual analog of stress (VAS-S), perceived stress scale (PSS), state trait anxiety inventory (STAI)], and (iii) general psychological health [general health questionnaire (GHQ), Beck depression inventory (BDI)]. All women were followed up for 12 weeks after the ET to determine clinical outcomes.
Main results and the role of chance: Fifty-two women successfully completed the study protocol. There were no significant differences between pregnant and non-pregnant women in the mean ± SD of the following: sAA levels (166,500 ± 115,498 versus 161,769 ± 122,482), FPI (143.7 ± 20.2 versus 136.7 ± 23.8), VAS-S (60.5 ± 21 versus 53.7 ± 24.9), VAS-P (8.6 ± 10.4 versus 8.52 ± 14.9), PSS (15.5 ± 4.8 versus 15.4 ± 3.7), STAI scores (41.2 ± 11.2 versus 42.7 ± 10.1), GHQ (25.5 ± 5.3 versus 26.7 ± 4.8).

However, there was a significant positive correlation between sAA and maternal age (Pearson Correlation 0.319, $p = 0.019$), FPI for social concern scores (Pearson Correlation 0.404, $p = 0.005$), FPI for relationship concern scores (Pearson Correlation 0.392, $p = 0.007$) and PSS scores (Pearson Correlation 0.314, $p = 0.023$).
Limitations, reasons for caution: Despite a strict study protocol for sAA sample collection, and the use of five comprehensive questionnaires, it is difficult to control for all the stressors that women undergoing ART may be exposed to, both related and unrelated to their treatment.
Wider implications of the findings: This is the first prospective observational study to examine the effect of psychological stress and biostress markers on pregnancy outcomes. Although it lacks of direct association with clinical outcomes, sAA appears to be significantly correlated to advancing maternal age, FPI scores for social and relationship concerns, and PSS scores.
Trial registration number: Not applicable.

P-511 Preferred roles in treatment decision-making, decisional conflict and depression: a longitudinal study of Chinese women making decisions on IVF treatment

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Study question: What is the relationship between decisional conflict and depression among women undergoing IVF treatment, and does role preference on treatment decision-making (TDM) moderate the relationship?
Summary answer: Decisional conflict at TDM led to higher-depression 3 months after decision, mediated by regret. Women preferring active TDM were less-depressed despite of conflict and regret.
What is known already: Infertility treatment is marked by open-ended nature, and patients need to decide whether to continue or terminate treatment when the current cycle fails. While there have been studies on treatment decision-making role preference among infertile women, little is known about the mental health consequences of such role preference across the time of treatment decision-making.
Study design, size, duration: A longitudinal study was conducted where infertile women indicated TDM preference at cycle failure (T0), decisional conflict when they decided on continuation/termination for next cycle (T1), and decision regret 3 months later (T2). They reported depression at 3 time points. 151 out of 246 women completed questionnaires (attrition rate: 39%). Mean age was 37.2, and they had had 1.12 cycles (range: 0–8) on average at the time of study. The study spanned for 2 years.
Participants/materials, setting, methods: The study population was comprised of Chinese female patients who failed to get pregnant in IVF treatment. They were recruited from a fertility clinic of a university-affiliated hospital in Hong Kong. Patients were approached after being notified about the negative pregnancy results. They were asked to complete scales on Fertility-Related Quality of Life and anxiety/depression across three time points and measures on role preference in treatment decision-making, decisional conflict, and regret at different time points.
Main results and the role of chance: Mediation analysis showed that the relationship between decisional conflict and depressed mood at T2 (controlling for depressed mood at T1) was mediated by decisional regret. Unstandardized direct effect between decisional conflict and depressed mood was 0.071 ($p < 0.001$), while indirect effect through mediation of decisional regret was found to be moderated by the role preference of treatment decision-making (TDM) (unstandardized indirect effect = 0.0002 and 0.0167 for passive and active TDM role preference respectively). Bootstrapping test confirmed that the mediation and moderation effects were statistically significant at 0.05 level. TDM role preference did not have significant effect on either decisional conflict or regret ($F = 0.10$ and 1.24 respectively, ns). Regret in IVF treatment can be interpreted as a consequence of preoccupation with comparing one's chosen treatment alternative to a second, non–chosen alternative. Decisional regret after making treatment-related decision becomes an important factor in addressing the psychological distress in terms of depression. Results have demonstrated another direction in healthcare communication regarding treatment decision-making.
Limitations, reasons for caution: Although there was no significant difference in age and education between those who completed the study and those who dropped out, self-selection bias remains a concern.
Wider implications of the findings: This study shows for the first time how treatment decision-making role preference leads to mental health consequences among women undergoing infertility treatment. Healthcare professionals should be aware of such preference and, when necessary, openly discuss issues of TDM conflict and possible regret regarding treatment decisions with clients.
Trial registration number: Nil.

P-512 Satisfaction with daily life and coping strategies in couple relationships and the psychoeducation of sexless couples (SLCs)

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Study question: Comparison of SLCs and a control group on satisfaction with daily and sex-lives, and the impact of stress, incorporating the results of psychoeducation for SLCs.
Summary answer: SLCs are not satisfied with their lives, lack of sex is therefore not a major problem. Psychoeducation is indicated as being effective in this situation.
What is known already: In general, Japanese couples do not consider that sex is an important part of a relationship. While this is a concept largely from social media, rather than from scientific research, the average low frequency of sex in Japan tends to support this assertion. Whenever SLC have been talked at first visit though, they say "We are satisfied with our daily life, and keep a good relationship." In fact, there is little evidence of a psychological impact on a couple's relation from the situation of no sex. However, this conflicts with the necessity to have sexual intercourse naturally to produce children.
Study design, size, duration: A questionnaire based survey was administered to 200 first visit couples seeking IVF intervention to have children at St Luke Clinic, from November 2014 to February 2015. Female patients were also given physical examinations, temperature checks, and tests for the state of their menstrual cycle. We also analyzed the frequency of sex. This is an important part of our research as it identified the sexless couples, along with the ethical considerations relating to fertility intervention.
Participants/materials, setting, methods: 200 couples were included in this investigation, which involved three kinds of questionnaire: the "GHQ-28 (General Health Questionnaire 28)," 4 items covering somatic symptoms, anxiety and insomnia, social dysfunction and severe depression; Kurosawa's "Relationship-Focused Coping for Couples (2013)," which includes 3 items for positive relations, concession and patience relations and avoidance relations; and an original questionnaire on their daily situation (22 items, about sex-lives, satisfaction, communication level, and so on).
Main results and the role of chance: 186 males and 172 females responded (response rate = 89%). Their ages were: wife (33.7 ± 4.5), husband (35.5 ± 5.7). Duration of marriage ranged from <166 months. Definition of "sexless" in this research is having sex less 1.5 times/month. This meant that 72 people (wife = 38, husband = 34) were classified as "sexless" couples. The reasons: for wives the problems were being more tired [physically ($t = 2.1$, $p < 0.05$), anxiety ($t = 2.2$, $p < 0.01$), and depression ($t = 0.6$, $p < 0.01$)] on GHQ-28, lower sexual desire (39.5%, $p < 0.01$), or a concern to have sex only to have a baby (47.1%, $p < 0.05$). SLCs were not satisfied with each other, and experienced much conflict (13.9%, $p < 0.05$). Thus, more SLCs indicated strongly that their level of sexual desire and motivation was low (26.4%, $p < 0.05$) than the control group (11.9%, $p < 0.05$). Also, SLCs do not have a positive physical relationship ($t = 18.1$, $p < 0.01$), tending to avoid ($t = 17.7$, $p < 0.05$) each other. Despite this, SLCs do not FEEL mental, or physical relationship problems (*n.s*). We stress the importance of sex at first visit, and we hold various types of seminars for patients as one of our policies, including counselling/psychoeducation for sexless couples. As a result, after 3 months the frequency of sexual intercourse increased to 3.8 times/month.
Limitations, reasons for caution: A couple's relationship and their social situation (busy) affects their sex-lives, but *sexless* may also be a mental condition. It is possible that their sex-life could recover if they have psychoeducation. However, physical sexual dysfunction may not recover using psychological means, and may be worse after IVF treatment is introduced.
Wider implications of the findings: SLCs coping ability on a number of personal dimensions is low. In this situation sex becomes work rather than pleasure. Also, SLCs do not communicate deeply even when discussing sex. We show that psychoeducation can increase the frequency of sexual intercourse even under IVF conditions, and improve their overall communication.
Trial registration number: N/A.

P-513 Depression and associated factors among Japanese women undergoing infertility treatment

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Study question: This study aimed to clarify the prevalence of depression among infertile women in Japan and identify factors associated with depression among these women.
Summary answer: The prevalence of depression was higher among infertile women than other women in Japan and the associated factors differed somewhat from previous studies.
What is known already: Many previous studies have reported on the psychological health of infertile women, particularly in regards to depression. However, how depression relates to sociodemographic factors (such as age and level of education) and infertility- or treatment-related factors (such as duration of treatment, cause of infertility, and experience with IVF-ET or ICSI) has not

been thoroughly investigated. In particular, age and duration of treatment have been found to have different effects on depression in different studies. Moreover, consistent results have not been obtained regarding the effects of the cause of infertility and experience with ART on depression.

Study design, size, duration: We conducted a quantitative, cross-sectional questionnaire survey between November 2011 and January 2012. Questionnaires were distributed to 300 Japanese women who were undergoing infertility treatment at one clinic in the Kanto region of Japan. The head doctor at the clinic distributed questionnaires directly to patients and those who agreed to participate were asked to return completed questionnaires by postal mail.

Participants/materials, setting, methods: Completed questionnaires were returned by 206 women (response rate, 68.7%). Responses from patients who did not have a child and who had not been diagnosed with recurrent pregnancy loss were analyzed. Independent variables included sociodemographic factors, factors related to infertility problems and treatment, subjective feelings of stress, and attitude about disclosing infertility. The dependent variable was the score on the Center for Epidemiologic Studies Depression Scale (CES-D). Data were statistically analyzed using SPSS version 11.0.

Main results and the role of chance: The mean CES-D score was 13.19 ± 9.56, and 36% of respondents exceeded the depression cut-off of 16.0. Level of education was negatively correlated with the CES-D score. Duration of infertility treatment, the number of hospitals visited, stress associated with sex life, stress associated with schedule adjustment for treatment, and stress associated with physical malfunction were found to have significant positive correlations with the CES-D score. As a result of one-way ANOVA, attitude about disclosing infertility was found to be significantly associated with the CES-D score. Furthermore, multiple regression analysis revealed that stress associated with sex life, stress associated with physical malfunction and attitude about disclosing infertility were significantly associated with the CES-D score. Age and level of education had significant negative association with the CES-D score. However, duration of infertility treatment, the number of hospitals visited, and stress associated with schedule adjustment for treatment were not associated with CES-D in this analysis. Experience with ART and the cause of infertility were not related to CES-D in any analysis. The mean CES-D score in this study was higher than that for women in the general population, domestically and internationally. Some factors associated with CES-D in this study differed somewhat from previous studies.

Limitations, reasons for caution: Caution should be exercised when generalizing the results of this study because the response rate was only 68.7% and only infertile patients visiting a clinic were included as subjects. Therefore, it is possible that patients with poor psychological health did not participate to this study.

Wider implications of the findings: The present results suggest that in order to effectively support infertile patients, it is important for medical staff to understand the psychological status of patients and their age, level of education and perceived stress and so on.

Trial registration number: The protocol of this study was approved by the ethics review board of Shukutoku University in 2011 (approval number: N10-002).

P-514 Psychosocial counselling in donor sperm treatment: the counsellors practices

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Study question: What do counsellors value in counselling intended parents in donor sperm treatment?

Summary answer: Counsellors combined screening and guidance, though they valued separate sessions. They raised different topics in different family types. Counsellors valued offering extended counselling after DST.

What is known already: In DST, counselling by qualified mental health counsellors is recommended to help intended parents in coping with the psychosocial implications of being a parent of donor-offspring. How counselors actually perform their counselling practices is unknown.

Study design, size, duration: We performed a qualitative study from January until September 2014 entailing 13 counsellors, who work in one of the eight Centres for Reproductive Medicine that offer DST in the Netherlands.

Participants/materials, setting, methods: We held one focus-group discussion with six counsellors and held seven individual semi-structured interviews with seven other counsellors. The focus-group discussion as well as the individual interviews were fully transcribed and analysed using the constant comparative method of grounded theory.

Main results and the role of chance: The counsellors had an average experience in counselling in DST of 8.2 years and had been trained as psychologist or as medical social worker. None had been specifically trained in DST counselling, but learned by practicing, by participating in congresses and from exchanging experiences with fellow counsellors. The policy of the clinics differed in whether or not offering counselling for heterosexual- and lesbian couples, but in all clinics but one counselling for single women was mandatory. The main topics that counsellors valued to discuss in all family types were assessment of mental health, stability of the relationship, future donor contact. Some counsellors valued to discuss in heterosexual and lesbian couples the position of the social parent. Especially in heterosexual couples they valued to discuss disclosure, and in single women their social network and economic situation. All counsellors offered intended parents extended counselling after DST, but experienced that when intended parents actually become parents, they seldom return for counselling. All counsellors felt that screening for eligibility and giving guidance and support in the same session restrained good guidance.

Limitations, reasons for caution: Limitations are the generalizability of our results, since only Dutch counsellors were studied and there is legal non-anonymity of sperm donors in the Netherlands, which is not common in all countries.

Wider implications of the findings: Our findings on what counsellors value in counselling in DST are a first step to contribute to achieve evidence based psychosocial counselling, which meets the need of (intended) parents. Whether parents wish any psychosocial guidance after treatment and childbirth and in what setting, is an essential issue needing further exploration.

Trial registration number: Not applicable.

P-515 Resilience influences on the outcome of IVF-ICSI

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Study question: Could women's Resilience have the capacity to improve IVF-ICSI outcomes ?

Summary answer: Higher resilience in infertile women undergoing IVF-ICSI cycles was positively correlated with women age and with Live Birth (LB) rate in IVF-ICSI cycles.

What is known already: Psychological resilience is an individual's capacity to prevent, minimize, or overcome stressful situations imposed by life adversity. Infertile women suffer from various psychosocial problems because of infertility and they adopt emotion-focused coping methods. Moreover IVF-ICSI treatments are characterized by high uncertainty and low personal control.

Resilient individuals feel confident they can overcome their emotions and resilience can be considered as an unspecific protective factor against infertility-specific distress. In addition adverse implications of couple's psychological distress for gamete biology and for IVF-ICSI outcomes has been suggested.

Study design, size, duration: A pilot cross sectional study of 75 women aged from 29 to 40 years during IVF-ICSI cycle was carried out between January and March of 2015 at the Asturias Central University Hospital (Oviedo, Spain) and *In Vitro* Fertilization Center of Asturias (CEFIVA), both affiliated with the University of Oviedo (Oviedo, Spain).

Participants/materials, setting, methods: Sixty-two infertile women were included in the study. Before starting the controlled ovarian hyperstimulation with a GnRH antagonist protocol, patients were asked to fill out an itemized general questionnaire (personal and partner sociodemographic data), the Connor Resilience Scale, the Center for Epidemiologic Studies Depression Scale (CESD-10)

and the 10 items Cohen Stress Scale. The research protocol of the study was reviewed and approved by the Asturias Ethical Committee, Oviedo, Spain.
Main results and the role of chance: Thirteen women decline to participate in the study or filled incomplete questionnaires, so the response rate was 82.7%. Oocyte retrieval mean was 8 ± 6.1 SD, fertilized oocytes mean was 5 ± 3.5 SD and single or double embryo fresh transfer were performed in 80% of cycles. Pregnancy rate was 36.8% and LB rate was 17.3%.

Total resilience values were positively correlated with women aged ($p = 0.02$). Mean resilience values were 32.4 ± 4.9 SD in women with LB and mean resilience values were 27.8 ± 4.8 SD in women with negative outcome ($p = 0.026$). In this sense, the probability of IVF-ICSI negative result was higher in women with lower resilience values OR: 2.4 (95% CI: 1.9–2.9).

Mean Cohen Stress Scale or CESD-10 questionnaires values did not show statistical differences in women with and without LB.
Limitations, reasons for caution: This is only a pilot study with a low statistical power due to the short sample size, so data must be managed carefully. Moreover the study design did not allow finding an exact explanation of the mechanism of action of resilience.
Wider implications of the findings: Our data partially agree with other research and suggest that identification of women with lower resilience values could be useful for planning the best IVF-ICSI strategy in order to obtain LB.
Trial registration number: N/A.

P-516 Self-efficacy to deal with infertility and its mediator role between anxiety, negative affect and impact on life domains

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Study question: Does the perception of self-efficacy to deal with infertility have a mediator role in the relationship between anxiety, negative affect and impact on life domains?
Summary answer: The perception of self-efficacy plays a mediator role on the association between anxiety, negative affect and impact on life domains.
What is known already: The emotional impact of infertility has been widely studied but factors that may have a protective role have deserved less attention. Anxiety symptoms and negative affect are common emotional responses associated with infertility related stress, particularly when considering the dimension of "impact on life domains." The perception of infertility self-efficacy involves cognitive, affective and motivational self-regulation processes and establishes the appropriate skills for facing demanding situations. Previous studies have shown that infertile patients who show high self-efficacy tend to present more positive emotional status, persist with medical treatment or achieve a family-building resolution.
Study design, size, duration: Cross sectional study. Data were collected through self-report instruments in 4 public infertility centers and 3 private clinics between June 2010 and September 2012. Inclusion criteria were age (18 years or older), and an infertility medical diagnosis. The study was conducted in a sample of 309 infertile patients pursuing medical treatment.
Participants/materials, setting, methods: Three hundred and nine infertile patients (262 women and 147 men) completed a set of standardized self-report measures: fertility Problem Inventory, Spielberger State Anxiety Inventory, Negative Affect Scale and Infertility Self-efficacy Scale. Patients were invited to participate by their medical doctors, signed a written informed consent and were asked to fill in the questionnaires at home. Participants were also requested to return the questionnaires to the research team by mail.
Main results and the role of chance: Correlation analyses show that anxiety, and negative affect are significantly associated with infertility self-efficacy and infertility-related stress, namely the dimension "impact in life domains." A mediation analysis was conducted to examine whether the perception of infertility self-efficacy mediated the effect of anxiety and negative affect on the impact in life domains. Statistical analyses were carried out using SPSS v. 22, and path analyses were estimated in AMOS (v. 22) with bootstrap procedures (2000 samples). The model explained 40% of the impact on life domains of the infertility-related stress variance and an excellent model fit was found with a non-significant chi-square of 1.161 (df = 1, $p = 0.281$). All the paths were statistically significant and the significance of indirect mediational paths was further confirmed using bootstrap resampling method. Results indicated that negative affect predicted greater impact on life domains directly with an effect of 0.18. There was also an indirect effect of negative affect on impact on life domains ($b = 0.20$, 95% CI = 0.13 to 0.28, $p = 0.001$) through infertility self-efficacy. Anxiety predicted elevated impact on life domains fully through diminished perception of infertility self-efficacy ($b = 0.13$, 95% CI = 0.06 to 0.21, $p = 0.001$).
Limitations, reasons for caution: Although path analysis is a powerful statistical technique our findings rely on cross-sectional and self-report data. This design limits robust causal conclusions to be drawn and points to the need of future replication studies. Additionally, participants were at different stages of medical treatment and this may add confounding variables.
Wider implications of the findings: Findings point that increasing the patients' confidence levels on their own cognitive, emotional and behavioral skills related to infertility may contribute to attenuate the influence that anxiety and negative affect may exert on the patients' life domains. The study emphasizes the importance of targeting and promoting self-efficacy in infertile patients.
Trial registration number: The study was not a trial.

P-517 Infertility impact on male and female sexuality: a comparative study

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Study question: Is infertility impact on sexuality the same for men and women?
Summary answer: When experiencing infertility, women sexuality was significantly more affected than men's.
What is known already: Previous studies have shown that women's sexuality is more affected than their partners' when they experience infertility. However, there was no data evaluating Brazilian couples.
Study design, size, duration: Cross sectional, descriptive study. Three hundred infertile patients were included (150 men and 150 women), between January 2015 and December 2015.
Participants/materials, setting, methods: A female sexual function score and a male sexual function score (both validated for Brazilian subjects) were used. Mann–Whitney test was used for statistical analysis of sexual function scores and Fisher's Exact Test was used to compare sexual performance. Adopted $p = 0.05$ statistically significant.
Main results and the role of chance: A statistically significant difference was found when comparing sexual scores between men (median = 81.6) and women (median = 75.2) ($p < 0.001$). When sexual performance was compared, 58.7% of men reported to have a good performance while 66.7% of women reported regular performance or null performance ($p < 0.001$). Furthermore, men had less sexual desire dysfunction (6.7 vs. 14.7%; $p = 0.025$), less sexual arousal dysfunction (0.7 vs. 12%; $p < 0.001$) and less orgasm dysfunction (0.7 vs. 20%; $p = 0.001$) than women.
Limitations, reasons for caution: More studies are needed, with larger samples, to confirm these findings.
Wider implications of the findings: Infertility impact on sexuality is more important in women than in men. Health care providers that deal with infertile couples must be aware and pay special attention to this condition.
Trial registration number: Not applicable.

P-518 Actor and partner effects between gender role attitudes and fertility-related quality of life (QoL) in dyads of infertile couples

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Study question: How are personal gender role attitudes connecting to the own and the partner's fertility-related quality of life in couples with fertility problems?

Summary answer: Instrumentality is associated mainly with own QoL for both members of the couple, while male and female partner's expressivity is beneficial only for the man.
What is known already: Previous studies suggest that gender role attitudes influence how infertile men and women cope with their fertility problems. Gender role attitudes relate to infertility stress: persons with instrumental and combined attitudes suffer less from distress caused by involuntary childlessness.
Study design, size, duration: A cross-sectional study performed from February 2012 until April 2014 in five Hungarian, one German and one Jordanian fertility centers. Couples were recruited who had primary or secondary infertility and had not been treated in the participating clinics before.
Participants/materials, setting, methods: The study sample consists of 375 couples (750 women and men) attending the first infertility consultation. They were invited by a medical assistant to fill out a questionnaire package containing the International Fertility Quality of Life Questionnaire (FertiQoL), Personal Attribute Questionnaire (PAQ) and sociodemographic questions. Data were analyzed with the Actor Partner Interdependence Model.
Main results and the role of chance: The average age of the participants is 34.4 years, their couple relationship lasts for 7.17 years and they have been wishing for a child for 3.23 years on average. A proportion of 45.8% of the participants have a higher education.

For both males and females, instrumentality has an actor effect on better global fertility-related quality of life ($ps < 0.05$). There is also a tendency for positive partner effects for both members' QoL. Fertility-related quality of life of the man is connected with their own and the female partner's expressivity ($ps < 0.05$). Expressivity of the woman has only a tendentious positive effect on her own QoL.
Limitations, reasons for caution: Cross-sectional studies are not eligible verify cause-effect relations between the examined variables so our results show only correlations between gender role attitudes and fertility-related QoL. We recommend prospective study designs to explore proper causality between personal attitudes and psychological reactions to infertility.
Wider implications of the findings: Our finding about the partner effect of female expressivity on male infertility-related QoL could be applied in the psychosocial counseling for couples with fertility problems. Counselors could help the couple to be aware of this connection and to utilize it.
Trial registration number: Not applicable.

P-519 Women's perceptions of acupuncture in relation to *in vitro* fertilisation: the body and the mind

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Study question: How do women having *In Vitro* Fertilisation treatment perceive the role of acupuncture?
Summary answer: Women perceived acupuncture to be preparing their body through physical changes and preparing their mind through psychological changes.
What is known already: Accumulative success in IVF is but relies on women "staying the distance" through repeated treatments. Women discontinue treatment due to the physical and psychological burden of repetitive stress. Also psychological stress is known to reduce pregnancy chance. Women are increasingly seeking acupuncture parallel to IVF. Few studies are able to report an understanding of why women seek acupuncture and what perception they hold about its impact but those studies previously undertaken report that women believe it will increase their chance for pregnancy by enhancing reproductive physiology or by reducing stress.
Study design, size, duration: This study took a qualitative approach, was nested within a Randomised Controlled Trial and interviewed 50 women individually between April 2012 and September 2014.
Participants/materials, setting, methods: 50 women were recruited from an RCT of acupuncture in IVF and interviewed individually. Interviews were semi-structured and sought information about the women's perceptions of infertility, IVF and acupuncture. Data was subjected to theoretical thematic analysis to identify the attitudes and beliefs of the participants on acupuncture, what it does, how it "works" and what it feels like.
Main results and the role of chance: Two major themes emerged in the data: preparing the body and preparing the mind. Acupuncture was perceived as having an impact to the body through physical changes in the nervous, circulatory and hormonal systems thereby creating higher quality eggs, embryos and endometrial receptivity. Acupuncture was also perceived as having an impact psychologically through relaxation, mindfulness, calmness, and peacefulness. Acupuncture required stillness and this allowed women time to be still and to reflect on the meaning of embryo transfer. Themes were validated according to qualitative research standards.
Limitations, reasons for caution: Participants were drawn from only five of the nine participating RCT recruitment centres. Despite aiming to recruit as diverse a sample as possible the sample was dominated by Caucasian, well-educated women.
Wider implications of the findings: This qualitative study provides insight and deeper understanding of how women think about acupuncture in relation to their bodies in the context of IVF and infertility, and provides some insight into their reasons for engaging with this therapy.
Trial registration number: The RCT in which this study was nested is registered with the Australian and New Zealand Clinical Trial Registry 2/3/2011 (see http://www.ANZCTR.org.au/ACTRN12611000226909.aspx). ACTRN12611000226909.

P-520 Effect of religiousness on anxiety and depression in infertile women

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Study question: To evaluate whether religiousness/spirituality affects symptoms of anxiety and depression in infertile women.
Summary answer: Religiousness/spirituality does not correlate with symptoms of anxiety/depression. However, there is a positive correlation between religiousness/spirituality and duration of infertility and number of previous treatments.
What is known already: A few studies show that religiousness/spirituality is important in coping and adaptation to infertility. However, there is no data using validated scores correlating religiousness with anxiety and depression.
Study design, size, duration: Descriptive cross-sectional study with 80 patients starting assisted reproduction treatment (timed intercourse, intrauterine insemination and *in vitro* fertilization). Data were collected from February 2015 to January 2016.
Participants/materials, setting, methods: Spirituality Self Rating Scale (SSRS) was used to evaluate religiousness/spirituality and Hospital Anxiety and Depression Scale (HADS) to evaluate anxiety and depression. Statistical analysis was performed using the Spearman correlation coefficient in the correlation of SSRS scores with anxiety and depression scores and also in correlation with the number of previous infertility treatments and duration of infertility.
Main results and the role of chance: SSRS scores do not correlate with anxiety scores ($s = 0.181$; $p = 0.108$) or depression scores ($s = 141$; $p = 0.214$). However, a significant increasing correlation between SSRS scores and duration of infertility ($s = 0.302$; $p = 0.006$) and number of previous treatments ($s = 0.330$; $p = 0.003$) was found.
Limitations, reasons for caution: Further research with larger samples and different populations are needed to confirm the results of this study.
Wider implications of the findings: Religiousness/spirituality does not seem to affect symptoms of anxiety and depression in infertile women. However, it might be an important resource in coping infertility, among patients experiencing long duration of infertility and multiple treatments for that disease.
Trial registration number: Not applicable.

P-521 Can patients verify their treatment process and its quality? Conclusions from patients monitoring over ART centres in Poland

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Study question: Do patients have a real influence on their process of treatment: how the "patients rights" are practised and perceived by medical staff in ART centres?
Summary answer: Apart from declarative understanding of patients' rights, Polish ART centres seem not to be willing to practice patients-oriented health care.
What is known already: Reproductive medicine realizes one of the modern medical regimes described widely in the literature (Foucault, 1975; Thompson, 2005). Pregnancy, the final goal of MAR therapy, may overshadow the serious social and personal costs being paid by the patients, i.e., submission to medical authority against their own beliefs and consent to being disciplined by medical regime. Enhancing patients' voices and input into medical processes can equalize the natural dissproportion in distribution of knowledge and power. However, in the post soviet societies the dialogue between medicine and social actors seems to be often delusional with no real impact on the practice.
Study design, size, duration: In 2014–2015 Polish patients' organisation "Our Stork" led the project titled "Patients monitoring over MAR centres" with the participation of 35 MAR centres, which were visited by 13 trained social auditors. The project was based on direct observation of ART centres' daily work and structured interwievs with medical staff and patients. The conclusions deriving from the project were presented in Polish Parliament in Autumn 2015.
Participants/materials, setting, methods: 35 Polish MAR centres were audited by 13 auditors from January 2014 to April 2015. Analyses were based on: 299 civil contracts presented by MAR centres, 89 interwievs with patients, 152 interviews with doctors, midwives, receptionists and the ART centres' owners, direct observations, and on the document titled "Patients guidelines on infertility" prepared by "Our Stork" Association with the participation of 722 questionnaired patients from the Internet forum (92 thousands of registered patients).
Main results and the role of chance: Three areas of discrepancy between ART centres declarations and presented guidelines, and patients' practice were distinguished: (1) Area of procedures concerning gamete and embryo donations (i.e., lack of counselling and psychological consultation; lack of the information about general rules applied; treating the process by medical staff as purely biomedical disregarding the social aspects of the process), (2) Area of patients' rights recognition [in 299 analysed ART centres' civil contracts, 228 abusive clauses, lesser errors in legal terms discriminating patients and children (n = 74) and/or violating patients' and human rights (n = 93) were found], (3) Area of communication with patients (publishing non transparent data applied to efficiency of ART centres with no methodology disclosed; discouraging patients to SET policy; using paternalistic formulas in materials and documents distributed by centres). Apart from official declarations and legal regulations, the true relations between patient and doctors seem to be organized around biodisciplining paradigm of "obedient patient."
Limitations, reasons for caution: Before launching the monitoring, 44 ART centres which offered IVF in past 10 years were mapped, two of which had ceased activity, and seven refused to participate. Therefore, 35 ART centres out of 44 were audited, and the conclusions referred to this number.
Wider implications of the findings: Including patients' perspective into the process of health care provisions may lead to improvement in quality of services and creation of more patients-oriented health care. One of the reasons for treatment discontinuation, is the lack of patients' trust in the way the ART centres operate on area of communication.
Trial registration number: Not applicable.

P-522 Do parents with offspring following identity-release donation put their disclosure intentions into practice? Follow-up of heterosexual couples with 7-year-old donor-conceived children

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Study question: Are disclosure intentions of heterosexual couples with children following identity-release oocyte and sperm donation put into practice?
Summary answer: The majority of parents complied with their disclosure intentions and had started the disclosing process when offspring had reached the age of 7.
What is known already: Donor-conceiving couples are increasingly recommended to share information about the donor conception with their child from an early age. While international studies report that heterosexual couples support openness and plan to disclose this information to their offspring, there is a lack of longitudinal studies investigating to what extent such disclosure intentions are put into practice.
Study design, size, duration: The present study is part of the prospective longitudinal "Swedish study on gamete donation" including all fertility clinics performing gamete donation in Sweden. Consecutive groups of recipient couples were recruited 2005–2008 and followed with individual questionnaires before and after treatment with oocyte and sperm from identity-release donors. The present study includes data from two follow-up assessments: when offspring were age 1–4 and when they had reached age 7, respectively.
Participants/materials, setting, methods: Heterosexual couples with donor-conceived children following oocyte donation (OD) and sperm donation (SD) that participated at two follow-up assessments. Among those who responded to the first follow-up, response rates for the second follow-up were 74% for OD-parents (48 of 65) and 75% for SD-parents (61 of 81). Disclosure intentions and practice were assessed individually with study-specific items. Data were analysed with chi^2-tests.
Main results and the role of chance: Among parents who at the first follow-up stated that they planned to disclose before offspring age 7 (n = 31), a majority (77%) had started the disclosure process but one in five had not realized their intention. All but one parent who had planned to disclose when the child asks questions about where babies come from (n = 11) had started disclosing at age 7. In contrast, among those who planned postponing disclosure until "the child understands" (n = 25) only one-third had started disclosing during the preschool period. In total, 66% of OD-parents and 60% of SD-parents had started the disclosure process with their offspring at age 7, a few (n = 4) were unsure or unwilling to disclose and the remaining planned to disclose in the future. There were no significant differences in disclosure practice at offspring age 7 between parents following oocyte and sperm donation.
Limitations, reasons for caution: There is risk of selection bias, with recipients preferring non-disclosure declining study participation or dropping out. Comparison of initial disclosure intentions between responders and non-responders to the second follow-up did not indicate such selection bias. Results are partly generalizable to heterosexual parents of children conceived with gametes from identity-release donors.
Wider implications of the findings: The present results suggest increasing compliance with the intentions of the Swedish legislation among parents of donor-conceived offspring. Couples building a family with donor gametes may benefit from opportunities to discuss when it is appropriate to start the disclosure process.
Trial registration number: N/A.

P-523 Stress and infertility: a pilot study measuring cortisol levels in the hair of infertile women

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Study question: Is hair cortisol increased in women with infertility as compared to control subjects without infertility?
Summary answer: There was no significant difference found in overall cortisol levels in hair samples between infertile women and controls.
What is known already: Studies have shown stress has an impact in many areas of physical health. Infertility related stress is also a well-characterized entity. Chronic stress may contribute to factors such as reduced fertility and even poor pregnancy outcomes. Biomarkers for measuring acute stress have been well established but finding a "gold standard" for the measurement of chronic stress proves more difficult. Hair cortisol sampling has emerged as a

novel method for examining stress over longer periods of time and may prove clinically useful in management strategies for stress reduction in the infertility population.

Study design, size, duration: Observational case–control study. Sixty women were enrolled from April 2013 until April 2014. Thirty patients who had not conceived within the past year and were seeking IVF treatment served as the cases. Thirty age matched patients with proven fertility acted as our controls. All study patients were assessed for overall stress using the Perceived Stress Scale (PSS). In addition, infertile women were given the Fertility Problem Inventory (FPI), a survey measuring perceived infertility-related stress.

Participants/materials, setting, methods: Hair samples were taken by our clinical coordinator using a standard protocol. Each patient's sample was divided into two sections (0–3 and 3–6 cm). Hair grows on average 1 cm per month, therefore, each segment corresponded to a 3-month cortisol sample. Cortisol measurements were then attained according to the standard protocol developed in our laboratory.

Main results and the role of chance: There was no significant difference in overall cortisol levels between infertile women and controls. However, there was a significant difference between the infertile group and the controls at segment 1 ($F = 4.299$, $P = 0.043$). Interestingly, the infertility group had significantly lower cortisol levels in segment 1 compared to the controls. Mean segment 1 (0–3 cm) and 2 (3–6 cm) cortisol levels (ng/g) in the infertility group were 111 ± 135 and 121 ± 194 respectively; and in the control group were 173 ± 231 and 112 ± 147 respectively.

There was no significant relationship found between PSS and cortisol levels in either group. The infertility group, however, reported significantly higher levels of stress than controls on the PSS ($t = 2.6$, $P = 0.012$).

There was also no significant relationship found between FPI Global scores and cortisol levels in the infertility population. On the FPI, 37% of the infertility group scored above the 84th percentile compared to fertility norms, correlating with "moderately high" infertility related stress. Of these, 3.3% had scores above the 98th percentile, indicating "very high" stress. In the infertility group, there was a significant but moderate correlation between the PSS total score and the FPI Global score ($r = 0.41$, $P = 0.025$).

Limitations, reasons for caution: Cortisol levels in both groups were highly variable as shown in the large standard deviations. The small sample size may mask the true differences found in cortisol levels between the control and infertile female patients.

Wider implications of the findings: These results suggest that cortisol levels in hair might not have a simple positive linear relationship with subjective stress as has been assumed and emphasizes the need to expand this body of research.

Trial registration number: REB #103692.

P-524 Motives and considerations regarding PGD in couples carrying a structural chromosomal abnormality – a quantitative analysis

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Study question: What are motives and considerations of couples carrying a structural chromosomal abnormality deciding on preimplantation genetic diagnosis (PGD)?

Summary answer: The main reason for couples carrying a structural chromosomal abnormality to choose PGD is to increase their chances of an ongoing pregnancy.

What is known already: PGD can be offered to couples carrying a structural chromosomal abnormality to increase the chance of an ongoing pregnancy and healthy offspring. From our previous qualitative analysis we learned all couples who opted for PGD had tried to conceive spontaneously. They considered PGD because of their experience (infertility, recurrent miscarriage, termination of pregnancy, affected child). A majority of couples indicated that an important reason for them to choose PGD is wanting to increase the chance of a successful pregnancy. The present study aimed to quantify couples' motives and considerations deciding on PGD in a larger sample.

Study design, size, duration: A quantitative cross-sectional survey study investigating the motives and considerations regarding PGD or other reproductive options of couples carrying a structural chromosomal abnormality. 119 couples were included between January and May 2015. They made their reproductive choice after extensive genetic counselling on PGD.

Participants/materials, setting, methods: Couples carrying a structural chromosomal abnormality who had an informative consultation in a licensed large PGD centre between 1996 and 2012 were invited to fill out an online questionnaire on demographic and clinical characteristics and possible motives, categorized as physical, psychological, social, ethical and practical, considering PGD. Univariate and multivariate analysis was performed to investigate the relationship between couples motives and their actual choice to opt for or refrain PGD.

Main results and the role of chance: The response rate was 72.8% (131 respondents). After excluding couples who were rejected for PGD treatment 119 respondents remained for analysis. 19.3% of participants used PND before they came for the informative PGD consultation. The majority of couples chose for PGD after the PGD consultation (78.2%). 41% of couples actually started PGD treatment. Only nine participants (7.6%) regretted the reproductive choice they made, either PGD or not PGD, after the informative consultation. Respondents with a higher educational level and those who had (strong) positive feelings regarding spontaneous conception were less likely to opt for PGD. Couples who have a strong wish to prevent (further) miscarriages opt for PGD more often. Partner who carried the chromosomal translocation, presence of subfertility, number of previous miscarriages, and parity were not associated with reproductive choice.

Limitations, reasons for caution: Due to the retrospective questionnaire there is a risk of recall- and information bias. A relatively large group changed their reproductive choice over time. After counselling 78% of couples opted for PGD, in time only 41% started. We learned from our previous study main reason is a spontaneous ongoing pregnancy.

Wider implications of the findings: Couples' choice to decide on PGD is very personal. Few of the investigated motives were significantly associated with couples' reproductive choice. This information learns us it is important to personalize counselling and psychological support during the decision making process for future couples carrying a structural chromosomal abnormality.

Trial registration number: N/A.

P-525 Breaking the news to children conceived by *in vitro* fertilization: how do parents behave?

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Study question: How parents deal with revealing to their children a mode of conception by *in vitro* fertilization (IVF)?

Summary answer: Most of parents intend to reveal to their offspring the use of IVF and many would appreciate the assistance of an informative and educational document.

What is known already: Announcing to offspring their mode of conception has been extensively studied after gamete donation or adoption. It is now commonly accepted that parents should reveal their child's origin as soon as possible because of the negative consequences of family secrets. Little is known on parental behaviour toward disclosure in case of intra-conjugal IVF. Nevertheless, despite the non missing genetic link, intra-conjugal IVF is different from a spontaneous pregnancy. Actually, many infertile patients, who needed IVF, can have negative thoughts like shame or guilt feelings, that in addition to secrecy within family, may have negative consequences on family fulfilment.

Study design, size, duration: Descriptive and prospective study from October 2013 to April 2015 in the Reproductive Centre of Bordeaux University Hospital (France).

Participants/materials, setting, methods: The study inclusion was proposed to parents who consulted for a second or third child and who already conceived by intra-conjugal IVF in the past, regardless the current age of the child(ren). If they agreed to participate, a survey questionnaire was given to both members of the couple. The participation was non-mandatory, individual, anonymous and free.

Main results and the role of chance: A total of 190 questionnaires was distributed to 95 couples, on which 92 were sent back, resulting in a response rate

of 48.4%. Thirteen per cent of parents (12/92) already revealed the mode of conception to their child(ren), 67% (62/92) thought about revealing it, 17% (15/92) were not yet decided and 3% (3/92) did not want to reveal. At the time of the survey, children born after IVF were between 9 months and 7 years old (median age: 3.2 years old). Most parents thought that the best time to disclose was "when we think our child will be able to understand"; (49%) or "when he will ask to us where do babies come from" (27%). Sixty-one percent of parents would like to dispose of a pedagogical tool. For 81% of them, the best educational material would be a children's book. Moreover, 31% of patients reported that IVF had been a psychological painful experience, with a feeling of personal failure and a sense of being different than other couples, although they gave birth to one or more children thanks to this technique.

Limitations, reasons for caution: Most people who came back for a second or third child and answered to the questionnaire had a masculine infertility (55%), which can be considered as a selection bias. As in studies about gamete donation, there could be a gap between questionnaire's answers (what people think to do) and reality.

Wider implications of the findings: Our study shows that many parents intend to reveal to their child(ren) recourse to IVF. We point out that there is undoubtedly a need for informative support. Moreover, because IVF is frequently experienced as a psychological injury, it seems essential to develop strategies to assist couples who face with infertility.

Trial registration number: Not applicable.

P-526 Impact of treatment decisions and significance of cultural beliefs in predicting quality of life of Chinese infertile women

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Study question: What is the impact of treatment decision-making on Chinese infertile women and what factors can predict their quality of life (QOL) 3 months after the decision?

Summary answer: QOL in mind-body, social and tolerability improved but relational, environment and mental-health worsened. Believing "infertility as violation of filial piety" was identified as major predictor.

What is known already: When engaging in treatment decision making, there has been a paradigm shift to look at a patient's decision not only based on its subsequently treatment outcome or disease prognosis, but also how the decision impacts on his/her quality life. In the case of infertility, the decision of continuing or discontinuing treatment is often embedded in a midst of socio-cultural considerations such as childbearing desire and cultural attitudes towards fertility, especially in societies who regard maternity as a central milestone in adult development and childlessness will cause major disruption in women's lives by inferring with the established and desired life course.

Study design, size, duration: A longitudinal questionnaire study was conducted with 151 Chinese infertile women. Data was collected on their quality of life (FertiQOL and HADS), childbearing desires, attitudes towards infertility, and demographic details at three time-points: notification of treatment failure (T0), immediately after treatment decision making (T1) and 3 months after (T2). The study spanned 2 years.

Participants/materials, setting, methods: Participants were recruited from a fertility clinic at a university affiliated public hospital. Out of465 patients approached by the research assistant, 246 agreed to participation, and 151 completed all questionnaire (attrition rate 39%). On average, their age is 37.2 years (SD 3.27), received subfertility diagnosis for 3.8 years and had experience 1.1 IVF treatment cycle prior to the current failure. They mostly received tertiary education, non-religious, and were full-time employed.

Main results and the role of chance: Between baseline (T0) and immediately after treatment decision making (T1), no significant changes were found in the women's quality of life domains and mental well-being, except a decrease in anxiety scores [$t(150) = 6.57$, $p < 0.001$]. Three months after the treatment decision (T2), significant improvement was found for the Mind-body [$t(150) = 3.17$, $p < 0.01$], Social [$t(150) = 10.8$, $p < 0.001$], and Tolerability [$t(150) = 5.34$, $p < 0.001$] domain. However, the scores for the Relational [$t(150) = -15.53$, $p < 0.001$] and Environment [$t(150) = -4.64$, $p < 0.001$] domain, as well as the anxiety [$t(150) = 13.96$, $p < 0.001$] and depression [$t(150) = 24.15$, $p < 0.001$] scores were worsened. Regression analysis revealed the participant's identification towards the cultural saying that "infertility is biggest violation to filial piety" is an effective predictor of her anxiety level ($\beta = 0.21$, $p < 0.01$) as well as her quality of life in the emotional ($\beta = -0.22$, $p < 0.05$), mind-body ($\beta = -0.18$, $p < 0.05$), and tolerability domain ($\beta = -0.20$, $p < 0.05$) 3 months after treatment decision making.

Limitations, reasons for caution: Data was collected from a single fertility clinic, so may not be representative of all IVF patients across clinics. Although there was no significant difference in age, education, and religious background between those who completed the study and those who dropped out, self-selection bias is a concern.

Wider implications of the findings: This study showed impact of infertility treatment decisions on women's quality of life and psychological adjustments. By identifying cultural beliefs as predictor of post-decision QOL, psychosocial support may consider exploration of such beliefs with the infertile couple prior to treatment, and allow re-visiting of these conversations throughout the treatment course.

Trial registration number: Nil.

P-527 Professionals' experiences with patients undergoing e-therapy, a cohort study

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Study question: What are the experiences of professionals in clinical fertility care with patients undergoing e-therapy?

Summary answer: Experiences with e-therapy were positive and it was considered useful. Before introducing e-therapy in daily clinical care, structured information should be provided to fertility staff.

What is known already: Internet-based interventions (e.g., e-therapy) are promising in reducing psychological distress. In fertility care e-therapy on psychological distress has been introduced, but the beneficial effects have to be evaluated. Furthermore, facilitators and barriers when introducing e-therapy in daily clinical practice have to be assessed. The experiences of professionals in clinical fertility care have not been evaluated before, and this could be crucial to enhance an effective introduction and implementation of e-therapy in daily clinical care.

Study design, size, duration: After conducting a prospective randomised controlled trial (RCT) aimed to reduce distress in women undergoing IVF treatment using an e-therapy program, we performed a survey to analyse the experiences of the participating professionals with patients undergoing e-therapy. A questionnaire was sent to a representative sample of fertility care professionals, i.e., nurses, physicians and secretaries ($n = 10$) and a structured interview was performed with the coaches who performed the e-therapy ($n = 2$).

Participants/materials, setting, methods: We included professionals working at the fertility care department and the coaches working at the psychology department who were involved in the RCT. In the first group questionnaires were sent, asking for professionals' experiences during the RCT, e.g., the provision of information or their time-investment. In the second group a structured interview was performed in which experiences with conducting the e-therapy were asked as well as suggestions for improvement. Results were analysed using descriptive statistics.

Main results and the role of chance: All fertility professionals stated they were well informed about the study, the e-therapy content and which patients participated. Nevertheless, 40% appreciated more information, preferable by a face-to-face presentation. All fertility professionals assessed e-therapy as useful. Assumed barriers for e-therapy, such as no face-to-face contact or Internet problems, were mentioned in 29% (all nurses). The time invested by the professionals themselves in the e-therapy program was considered as minimal.

The psychology professionals assessed the assignments as useful, with exception of the stress relaxation assignment. They suggested involving the partner during the e-therapy. The mean score of the professionals was comparable with the previously evaluated patients' score (mean score 3.73 out of 6 versus 3.79). Although the coaches preferred face-to-face contact, they assumed Internet contact could be able to replace this in specific circumstances. They grade

the program as positive and would recommend it to other professionals (mean score 7.5 out of 10 versus 6.6 in patients). When introducing e-therapy in daily clinical care, the involvement of a coach and the intake meeting with face-to-face contact were assessed as important.
Limitations, reasons for caution: The experiences of professionals could be assessed only in this centre, because e-therapy was evaluated in a single centre RCT. Generalisability could be improved by expanding e-therapy to other centres. Although we used a representative sample of clinical professionals, the uptake rate in both physicians as nurses could be higher.
Wider implications of the findings: In fertility care, this is the first study that has evaluated the experiences of professionals with patients undergoing e-therapy. Important information on professionals' experiences was obtained. This knowledge could be useful in introduction and implementation of e-therapy initiatives in daily clinical care.
Trial registration number: ClinicalTrials.gov NCT 01283607.

P-528 Mental health of mothers and fathers after successful oocyte donation treatment in pregnancy and early parenthood: a 1-year prospective study

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Study question: Is psychological wellbeing of mothers and fathers after oocyte donation (OD) treatment similar to parents after IVF/ICSI with own gametes and natural conception (NC)?
Summary answer: OD mothers showed less sleeping difficulties and symptoms of social dysfunction compared with IVF/ICSI and NC controls whereas no differences were found among fathers.
What is known already: Absence or low levels of parental mental health symptoms (depression, anxiety, sleeping difficulties and social dysfunction) have been found to be beneficial to children's psychosocial well-being. Much of the research has focused on the experience of parenting a child conceived through OD treatment. Studies have shown that the relationship with the child is comparable among couples who had undergone IVF/ICSI with own gametes and naturally conceived (NC) controls. Furthermore, studies of parents marital relations do not show differences between these groups. No data exists on OD mothers' and their partners' mental health during pregnancy and early parenthood.
Study design, size, duration: This is a prospective, longitudinal questionnaire study of couples conceived with OD treatment in five fertility clinics in Finland. The OD group consisted of 26 mothers and their matched IVF/ICSI ($n = 52$) and SC ($n = 52$) controls. The mothers and fathers filled-in the General Health Questionnaire (GHQ-36) questionnaires separately at gestational weeks 18–20 (T1), 2 months after the childbirth (T2) and when the child was 1 year old (T3).
Participants/materials, setting, methods: Matching to find controls was performed according to mother's age (≤34, 35–37 and ≥38 years), parity (primipara or multipara), type of pregnancy (singleton or twin pregnancy) and number of returned questionnaires. Full response rate (T1–T3) for OD mothers was 76.9% and for OD fathers 73.1%. The levels of mental health symptoms were compared between the OD, IVF/ICSI and NC group by MANOVAs, with Bonferroni post-hoc analyses separately for mothers and fathers.
Main results and the role of chance: At T1, the OD mothers showed less sleeping difficulties than the IVF/ICSI and NC women ($F = 3.116$, $p < 0.05$), but no significant differences were found in symptoms of depression, anxiety and social dysfunction. At T2, the OD women reported less anxiety ($F = 4.328$, $p < 0.05$) than the NC controls, less sleeping difficulties ($F = 4.358$, $p < 0.05$) and less social dysfunction ($F = 7.112$, $p < 0.005$) than the IVF/ICSI and NC controls whereas no group differences were found in depressive symptoms. One year after the delivery (T3), the OD women had again less sleeping difficulties ($F = 3.695$, $p < 0.05$) and less symptoms of social dysfunction ($F = 3.498$, $p < 0.05$) than the IVF/ICSI and NC controls but similar levels of anxiety and depression symptoms. Mental health symptoms of OD fathers did not differ from that of IVF/ICSI and NC control fathers at any time point.
Limitations, reasons for caution: The number of participating OD mothers and fathers was low and results are based on self-reported mental health. The couples in the OD group had received more psychological counselling before the pregnancy than the controls which may have improved their psychological readiness for parenthood and thus also mental health.
Wider implications of the findings: According to our small study, OD couples do not need more support in pregnancy and early parenthood compared with IVF/ICSI couples and naturally conceived couples. However, OD couples' mental health symptoms should be examined when there are child-related or social stressors in their life.
Trial registration number: None.

P-529 Identity-release egg donation in the UK: parents' disclosure decisions and thoughts about the donor

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Study question: Do parents who have conceived using open-identity egg donation intend to disclose this information to their children? How do they feel about the donor?
Summary answer: Most parents intended to disclose, although they did not want to know the donor's identity and felt ambivalent about the child knowing in the future.
What is known already: Less is known about egg recipient families than about families created through sperm donation. Disclosure rates amongst families in the UK with children conceived through anonymous egg donation are approximately 45%. However, nothing is known about disclosure intentions of families in the UK formed following identity-release egg donation. To date, only one European study has assessed disclosure intentions of identity-release egg recipient parents (in Sweden), and suggests that disclosure intentions amongst this group may be higher. The present study is the first study of UK families with children conceived through egg donation following the removal of donor anonymity in 2005.
Study design, size, duration: In-depth semi-structured interviews were carried out with 73 families who had a child following successful treatment with identity-release egg donation in the UK. Data were collected between December 2013 and June 2015, and form part of a larger study examining family functioning in families with infants conceived through egg donation.
Participants/materials, setting, methods: In-depth, face-to-face interviews were conducted with 73 heterosexual UK couples who had a child through identity-release egg donation. Mothers and fathers were interviewed separately (73 mothers, 57 fathers). The average age of the infants was 11 months. Data were obtained on whether or not parents intended to tell their child about their donor conception, whether they had told their family and friends about the treatment, and their thoughts about identity-release donation more broadly.
Main results and the role of chance: The majority of parents (75% of mothers, 82% of fathers) in the present study intended to tell their child about their donor conception. Eighty percent of mothers and 74% of fathers had told their own parents about the egg donation, with slightly lower numbers informing their siblings. Of the 18 families who had older naturally conceived children, half had told the older child about the donor conception. Most (83%) mothers had told more than one friend about the egg donation, although 20% expressed regrets about who they had told. The majority of couples (90%) had received counselling prior to treatment, and most had been advised to some extent by the counsellor to disclose the egg donation to the child. With regard to the donor, 69% of mothers and 62% of fathers reported a change in the frequency in which they thought about the donor since the child's birth. Most parents (40% of mothers, 57% of fathers) did not want to know the donor's identity in the future, and a further third were unsure. Just under half (43%) of the mothers in the sample expressed ambivalent feelings about the idea of the child knowing the donor's identity in the future.
Limitations, reasons for caution: There is a risk of selection bias, with families preferring non-disclosure perhaps less likely to participate in research, although this sample was recruited systematically through fertility clinics in the UK. Previous research with couples using anonymous gamete donation suggests that parents' disclosure decisions may change over time.
Wider implications of the findings: The findings suggest a trend towards greater openness in the UK amongst egg recipient heterosexual couples. That many parents raised concerns about the identity-release process in the longer term is of relevance to clinics and professionals supporting patients before, during and after treatment.
Trial registration number: N/A.

P-530 Congruence between partners' need for parenthood and its relationship to their quality of life: a dyadic approach between infertile couples

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Study question: Does the congruence (i.e., agreement or discrepancy) between infertile partners' need for parenthood (NP) impact on their fertility-related Quality of Life (QoL)?
Summary answer: The increase in the need for parenthood discrepancy between men and women is related to decreasing levels of their QoL.
What is known already: Research has consistently demonstrated that dealing with infertility and Assisted Reproductive Treatment (ART) significantly impairs individuals' QoL, measured by the gold standard Fertility Quality of Life tool (FertiQoL); and that the difficulties experienced by infertile subjects are exacerbated by their attitudes towards childlessness. Moreover, recent studies have highlighted the reciprocal influence between partners undergoing ART, showing that the NP varies within the couple. However, the effects of agreement or discrepancy between partners' NP are understudied. No previous studies on FertiQoL investigated whether partners who do not agree on NP are more likely to report a decreasing QoL, during ART.
Study design, size, duration: A cross-sectional study was conducted on 520 subjects (both parties in 260 infertile couples) consecutively referred for an ART, from February 2013 to February 2015.

Polynomial regression with Response Surface Analysis (RSA) was used to examine the extent to which the couples' combination (i.e., agreement or discrepancy) of women's and men's need for parenthood (independent variables) is related to their own QoL (criterion variables).
Participants/materials, setting, methods: Both parties in couples undergoing an ART (IUI or IVF) at the ANDROS Day Surgery Clinic, Reproductive Medicine Unit in Palermo (Italy) filled in the following questionnaires before starting their treatment: the "Need for Parenthood" subscale of the Fertility Problem Inventory (FPI) and the Fertility Quality of Life tool (FertiQoL).
Main results and the role of chance: Results of polynomial regression analysis show that partners' need for parenthood accounted for 19 and 17% of variance in women and men QoL, respectively. Women's needs for parenthood [$b = -0.52$, SE = 0.07, $t(15) = -7.41$, $p < 0.001$] and the squared term for this [$b = -0.20$, SE = 0.07, $t(15) = -2.82$, $p < 0.01$] were negatively related to their QoL. Men's needs for parenthood [$b = -0.37$, SE = 0.07, $t(15) = -5.19$, $p < 0.001$] and the squared term for this [$b = -0.18$, SE = 0.08, $t(15) = -2.24$, $p < 0.05$] were negatively related to their QoL. Coefficients of the Response Surface Analysis along the line of agreement showed that if the need for parenthood is congruent and rising, both partners' QoLs decrease ($a_1 = -6.26$, $p < 0.001$ and $a_1 = -5.14$, $p < 0.01$ for women and men QoL, respectively). Moreover, the curvature along the line of disagreement shows that both partners' QoL decreases as the discrepancy between partners' need for parenthood increases ($a_4 = -4.70$, $p < 0.05$ and $a_4 = -3.79$, $p < 0.05$ for women's and men's QoL, respectively). Finally, for both partners, QoL is lower when the direction of discrepancy is such that their own need for parenthood is higher than the other partner's ($a_3 = -10.03$, $p < 0.001$ and $a_3 = 3.54$, $p < 0.001$ for female and male QoL, respectively).
Limitations, reasons for caution: There are some limitations in this study: firstly, data were obtained from one only clinical site and secondly, the cross-sectional design of the study does not allow us to explain the relationships in terms of causality.
Wider implications of the findings: Given the relationship between the NP discrepancy and QoL, specific counselling for couples undergoing ART should be considered. In order to improve their QoL, counselling should be focused on balancing individual reasons for conceiving and on achieving a congruence between partners' underlying motivations for having a child through ART.
Trial registration number: Not necessary.

P-531 The anxious 14 days – the role of spirituality in helping women during their *in vitro* fertilization result awaiting period by a self-help I-BMS model (RCT)

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Study question: How effective is the I-BMS self-help model in alleviating the anxiety of the women during their IVF result awaiting period?
Summary answer: The self-help model was proven effective in alleviating women's anxiety, while the role of spirituality was also an important factor to their childbearing belief.
What is known already: Many women experience complicated emotions throughout IVF treatment. Among all treatment stages, many studies have proven that their uncertainty and anxiety level peaks in the waiting period between embryo transfer and pregnancy test. The situation is especially difficult and stressful for Chinese women who bear the obligation to extend the family tree.
The introduced model adopted Traditional Chinese Medicine (TCM) as the framework aims at maintaining a harmonious balance in holistic wellbeing. It has been proven effective in alleviating anxiety, improving marital satisfaction and achieving holistic well-being of the Chinese women in face of IVF treatment.
Study design, size, duration: The study has been launched since January, 2015. In our pilot study, 38 women who were going to undergo IVF treatment were recruited in an infertility clinic in Hong Kong, and were randomly assigned to three groups: spiritual–behavioral (SB) group ($n = 19$) included a 3-h I-BMS workshop and a self-help exercise book on exercises and spiritual stories for home practicing; Spiritual (S) group ($n = 10$) received the latter with only spiritual stories; Control group ($n = 8$) received educational materials on healthy diet.
Participants/materials, setting, methods: 38 women who were going to receive IVF treatment participated in the study. They were invited to complete a set of self-administered questionnaires which comprised of Chinese State-trait Anxiety Inventory (C-STAI) and Importance of Childbearing Index on the day of embryo transfer (T1) and the day of pregnancy test (T2). The results were analyzed by ANOVA.
Main results and the role of chance: The mean age of the participants was 38.14 years old (SD: 2.26). They had married for 7.25 years (SD: 3.12) and had gone through 2.33 treatment cycles (SD: 1.45) on average. 11 out of 38 participants had experienced at least 1 previous pregnancy loss. The course of subfertility was mostly due to male factors ($n = 14$), followed by unexplained factors ($n = 5$) and female factors ($n = 3$). Some couples faced both male and female factors ($n = 2$), while some did not indicate.

Participants in SB-group showed reduction in anxiety level ($T1 = 88.50 + 14.14$, $T2 = 87.84 + 15.76$, $p < 0.001$) as measured by C-STAI in pre–post test. However, the anxiety level of the participants in S group raised ($T1 = 86.50 + 24.92$, $T2 = 88.90 + 26.42$, $p < 0.001$), while that of control group increased the most obviously ($T1 = 89.14 + 21.97$, $T2 = 92.43$, $p < 0.001$).

Apart from the anxiety level, participants in S group showed significant reduction in their childbearing belief ($T1 = 17.29 + 3.20$, $T2 = 16.56 + 3.90$, $p < 0.001$) as measure by Importance of Childbearing Index, while SB group showed slight reduction ($T1 = 15.69 + 5.04$, $T2 = 15.47 + 4.78$, $p < 0.001$). In contrast, the control group demonstrated increased childbearing belief ($T1 = 14.63 + 4.87$, $T2 = 15.63 + 7.03$, $p < 0.001$).
Limitations, reasons for caution: The sample size was limited and might not be able to generalize. Moreover, some women who experienced earlier symptoms or signs of treatment failure, such as menstruation came before pregnancy test might bring external effect to the result. Their opinions towards to model during the period were unknown.
Wider implications of the findings: The self-help intervention allows more autonomy, flexibility and privacy at lower cost to fulfill the women's special needs during the result awaiting period. It could psychologically and physically prepare them to be ready for the treatment outcomes. With limited existing psychosocial services specific for this period, it could provide another choice of support.
Trial registration number: N/A.

P-532 Inflammatory markers in depressed pregnant women

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Study question: Is there a relationship between depression and anxiety levels and inflammatory markers, in pregnant women?
Summary answer: Our findings support that depression in pregnant women may be related with inflammatory process.
What is known already: In the literature, there are limited number of studies investigating the relation between depression and inflammatory markers, including cytokines TNF alfa, and IL-6. In this study, we aimed to examine the association between TNF alfa, and IL-6 levels and depression, and anxiety levels in pregnant women.
Study design, size, duration: This is a cross-sectional study with a sample of 145 pregnant in their 28–36 weeks. The current study was conducted in the Department of Obstetrics and Gynecology in an university hospital between 2013 October and 2014 November.
Participants/materials, setting, methods: We studied the plasma TNF alfa, and IL-6 levels of 145 pregnant. Inclusion criteria were (1) Being between 18 and 35 years old, (2) Having no history of psychiatric disorder. All participants filled out Prenatal Maternal Attachment Inventory, Edinburg Postpartum Depression Scale, State and Trait Anxiety Inventory. Relationships between variables was assessed by Pearson's correlation analysis. Predictors of the attachment levels and depression scores were examined by linear regression analysis. Significance at $p < 0.05$ were considered.
Main results and the role of chance: The mean age of the participants was 23.2 ± 4.5 years and mean gestation week was 32.9 ± 2.7. The group was divided into two groups, according to EPDS, Group I (EPDS ≥12) was considered as probable depression and Group II (EPDS <12) was as without depression. There were no difference between the two groups in terms of age, education, and gestational age. Women with depression had significantly higher TNF alfa, and IL-6 levels than non-depressive group.
Limitations, reasons for caution: Although we had a large sample the main limitation was we relied on self-reports instead of structured interviews. We did not consider the psychological features of fathers.
Wider implications of the findings: This study showed that 12% of the women during their trimester had probable depression. Depression had a negative relation on maternal–fetal attachment. TNF alfa, and IL-6 levels were the predictor of identifying depressive symptoms during pregnancy.
Trial registration number: This study was founded by Necmenttin Erbakan University Scientific Research Foundation. Trial registration number: 131218036.

P-533 "To whom childbearing is important?": the association between perceived childbearing importance, attitudes towards assisted reproductive technologies and psychological well-being among Chinese lesbians

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Study question: What is the impact of perceived importance of childbearing and attitudes towards assisted reproductive technologies (ART) on the psychological well-being of Chinese lesbians?
Summary answer: Chinese lesbians who perceived childbearing as important to their parents or their partners but did not want to seek ART reported higher anxiety level.
What is known already: Existing research in the West shows that lesbians tend to express less childbearing desire and are less likely to become parents compared with heterosexual counterparts. For involuntarily childless couples in general, fertility treatment has implications on psychological well-being and marital relationship, as it is considered a deviation from traditional family formation. For lesbians in particular, anxiety is associated with contextual factors, namely legal recognition of parental status and neighbourhood climate. Little is known about lesbians' perceived childbearing importance, attitudes towards ART, and psychological well-being in Asia, where heteronormativity remains dominant and access to ART is restricted to heterosexual couples.
Study design, size, duration: An online cross-sectional survey of Chinese lesbians in Hong Kong was conducted from December 2014 to March 2015. A total of 438 self-identified lesbians participated in the study.
Participants/materials, setting, methods: A total of 438 Chinese lesbians in Hong Kong were recruited by bulk e-mail invitations through local lesbian, gay, bisexual, and transgender organizations and a university in Hong Kong. They completed the online questionnaire which consisted of the Childbearing Importance Index, the Hospital Anxiety and Depression Scale, and other measures and questions related to their attitudes towards ART.
Main results and the role of chance: Compared to heterosexual childless women, Chinese lesbians in our study thought childbearing was significantly less important (3.30 versus 6.00 on a 1–10 scale, $t = 14.6$). Perceived childbearing importance was also negatively associated with age ($r = -0.23$), relationship length ($r = -0.18$) and full-time employment ($F = 4.29$). Vast majority of participants (92%) supported legalizing same-sex couple's access to ART, although less than half (41%) wanted to use it themselves to have children. Among those who thought childbearing was important to their parents or their partners, not wanting ART was associated with higher anxiety level. These results were significant at $p < 0.05$.
Limitations, reasons for caution: As the lesbian community remains a hidden population in Hong Kong, snowball sampling method and anonymous online questionnaire were used, rendering it difficult to ascertain the response rate and warranting caution when generalizing the results. The cross-sectional design also limited the ability to make causal arguments regarding the observed relationships.
Wider implications of the findings: This is the first quantitative study of reproductive preferences among lesbians in Asia. The findings are helpful for healthcare professionals to address the psychological burden of Asian lesbians in relation to reproductive issues, particularly those pertaining to their perceived expectations from parents and partners concerning childbearing.
Trial registration number: N/A.

P-534 The doctor–couple communication in reproductive medicine: a pilot study on actual assisted reproductive technology (ART) visits

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Study question: To explore communicational characteristics in ART visits and their association with patients' activation, patients' satisfaction and patients' adherence.
Summary answer: ART visits seemed highly patient-centered; patients seemed very active and very satisfied. However an association with specific verbal contents of the visit was not found.
What is known already: Communicational and relational aspects in reproductive medicine seem to be crucial for clinical decision making, retention in care and critical conversations with couples because of treatment low possibility of success. However, no studies have been realized regarding the actual interaction between the doctor and the couple in this medical context.

Study design, size, duration: A descriptive cross sectional study involving 9 Italian ART clinics where 28 clinicians and 175 patients (80 couples) signed a written consensus to be videotaped during their ART consultations. A total of 95 ART visits were collected in the period June 2014 to January 2015. 90 visits (51 first visits and 39 follow up visits) were eligible for the analysis.
Participants/materials, setting, methods: Patients filled out: before the visit the Patient Activation Measure (PAM) adapted for ART context (5-point Likert scale from 1 = totally disagree to 5 = totally agree), and after a satisfaction questionnaire (SATQ) (5-point Likert scale from 1 = poor to 5 = excellent). A 3-month telephone follow-up explored the adherence to clinician recommendations. The communication content was coded using the Roter Interaction Analysis System (RIAS), a validated and widely used coding system for categorizing verbal exchanges in the physician–patient interaction.
Main results and the role of chance: The response rate was 62.1%. Preliminary results refer to 30% of the visits. Both females and males reported high scores on the PAM (respectively $\mu = 4.25 \pm 0.38$ and $\mu = 4.21 \pm 0.43$) and on the SATQ (respectively $\mu = 4.45 \pm 0.49$ and $\mu = 4.29 \pm 0.61$). 85% of the couples declared after 3 months to have followed the clinicians recommendations; 17% of the couples declared to have asked an opinion to another ART center. As far as the communication content: physicians contributed for 63% and patients contributed for 37% of all consultation statements. The RIAS categories distributions for physicians and patients were respectively: biomedical questions (7%; 3.7%), lifestyle/psychosocial questions (1.7%; 0.8%), biomedical information (45.4%; 32.9%); lifestyle/psychosocial information (4.8%; 11.7%), emotional expressions (7.4%; 4.4%); facilitation and patient activation (13.4%; 5.7%), positive rapport (7.4%; 30.9%), negative talk (1.3%; 3.1%), social talk (0.7%; 1%), procedural talk (10.1%; 3.2%), unintelligible statements (0.8%; 2.6%). The patient-centeredness mean score was 2.5 (SD = 1.4). No correlations between physicians and patients RIAS categories and patients self-reported measures (PAM and SATQ) were found except for a positive correlation between total talk and SATQ for females ($r = 0.472$, $p = 0.008$) and a negative correlation between PAM and patient-centeredness score for males ($r = -0.462$, $p = 0.017$).
Limitations, reasons for caution: The results are preliminary and referred to the Italian context. A selection bias could be present both for accepting patients and physicians.
Wider implications of the findings: Results will allow a deeper understanding of the complexity of doctor–patient communication during ART visits, in particular concerning the engagement of the patient/couple during the encounter and its outcomes; results will be used also for tailoring the communicational training of the multi-professional team involved in reproductive medicine.
Trial registration number: The study protocol was approved by the Institutional Review Board (IRB) of the University of Milan (number 50/13) and by the IRB of the ART Centers.

P-535 Children's adjustment and perspectives in solo mother families

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Study question: How do children think, feel and fare in families formed by single women using donor sperm?
Summary answer: Although children in solo mother families are generally well-adjusted and report feeling positively about family life and friendships, they have questions about father absence.
What is known already: The existing literature has identified that the adjustment of very young infants raised in solo mother families is comparable to infants in heterosexual two-parent families. However, little is known about children's experiences as they grow older. This multi-informant, multi-method study is the first to systematically examine child adjustment and children's perspectives at the age at which children are old enough to understand their family circumstances and what it means to grow up without a father. It is the only study to have elicited children's own reports about their social and familial experiences.
Study design, size, duration: A cross-sectional study of 51 solo mother families and a comparison group of 52 heterosexual two-parent families with at least one donor-conceived child aged 4–9 years. Participants were recruited from a large UK fertility clinic that routinely treats single women. Participating families were matched in terms of the age and gender of the target child, and on demographic factors including mother's educational level, perceived financial difficulties, and psychiatric problems in the last year.
Participants/materials, setting, methods: A response rate of 72% was obtained. All mothers were administered standardized questionnaires of child adjustment and parenting stress. Solo mothers completed a semi-structured interview which asked about their children's feelings about father absence, and whether or not this was a topic of family discussion. Within the solo mother families, 47 children agreed to be interviewed, 25 (53%) of whom were girls, and 22 (47%) boys. Children were asked about family life and friendships.
Main results and the role of chance: Data were analysed both quantitatively and qualitatively. There was no significant difference between family types for total scores on the child adjustment questionnaire measure. Within the solo mother families, higher levels of financial difficulties [$F(2, 41) = 5.00$, $p = 0.01$] and higher levels of parenting stress [$F(7, 36) = 2.55$, $p = 0.03$] were each associated with higher levels of child adjustment problems. Mothers mostly reported that their children had neutral (39%) or mixed (28%) feelings about father absence, and qualitative analyses of mothers' reports indicated conversations about fathers to be a prominent feature of family life. However, most children (89%) who answered a question about changing their family circumstances either expressed a desire for trivial changes (38%) or no change (51%). Children mostly (59%) reported high (19%) or very high (40%) levels of enjoyment of school. All reported having at least one friend, and most (51%) named five or more friends. The majority (63%) had not been teased at school, or had experienced trivial teasing (34%). Preliminary findings from a current follow-up study of these children in middle childhood, specifically focusing on their thoughts and feelings about their donor conception, will also be reported.
Limitations, reasons for caution: Differences between family types may not have been identified owing to the relatively small sample sizes. Not all children responded to all questions asked, possibly owing to age-related comprehension ability.
Wider implications of the findings: Findings from both mothers and children indicate that at the age at which children begin to understand their family circumstances, they continue to function well. The study offers new insights into children's perspectives and experiences in solo mother families, and the processes that determine their adjustment, as they grow older.
Trial registration number: N/A.

P-536 The decision-making process after a failed treatment cycle: understanding compliance during fertility treatment

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Study question: What characterises the decision-making process about doing more treatment after a failed cycle?
Summary answer: Patients start treatment determined to follow all the recommendations. After failure, they do not re-negotiate the decision to stop treatment and strive to remain engaged.
What is known already: After a failed cycle, couples have to decide whether to continue undergoing treatment. Previous studies shown that 22% of patients do not comply and also that compliance rates decrease with treatment failure and the main reasons for this are postponement of treatment and psychological burden (Gameiro et al., 2012). Even if the main reasons are already identified, it still remains unclear how the process of decision-making happens, who is involved in this decision and what type of influence each member of the couple might have. This knowledge is useful to provide decisional-aid.
Study design, size, duration: Cross sectional qualitative study. Data was collected in August and December 2014. One-hour semi-structured interviews were carried out separately with the two members of the participating couples. The interview' analyses were performed by two independent coders based on Interpretative Phenomenological Analysis (IPA). The data was checked for credibility, which is an effort in qualitative research to ensure trustworthy findings (Elliot et al., 1999) by presenting results to other infertility and self-regulation experts for feedback.
Participants/materials, setting, methods: Purposive sampling was used to select participants. Participants were eight Portuguese heterosexual couples who experienced a failed cycle of IVF or ICSI between 6 weeks and 1 year prior to the interview, were deciding whether or not to do another treatment cycle, and were not advised to discontinue treatment. The main subjects of the interview schedule were parenthood goal importance, failed treatment experience and individual and couple strategies to deal with it.

Main results and the role of chance: Most couples decide to do all the treatment cycles recommended at the beginning of treatment. Therefore, after experiencing a failed cycle, individuals wanted to undergo more treatment regardless of the medical prognosis for the next cycle and largely due to the high importance they attributed to parenthood. Giving up on treatment was not an option. Consequently, patients used several strategies to self-motivate to remain in treatment, such as considering each treatment cycle as a learning opportunity to acquire new skills and to avoid future regrets and, seeing the treatment process as a "game of probabilities" that required persistence to get results. A lack of communication characterized the couples as men tended to voluntarily leave the decision-making to their partners, given that women endure most of the medical procedures. Despite this, couples recognize the role partners have in the regulatory process, mostly in terms of support provision. Some patients made the decision to do more treatment in the service of secondary goals (e.g., for the partner to be happy) instead of the primary parenthood goal.
Limitations, reasons for caution: Heterogeneity is accounted for by the existence of inter-sample differences between couples who have recently started their infertility journey and those who have had multiple failed cycles. However, all participants lived in the same country and decision-making processes may reflect Portuguese cultural values and social norms instead of universal ones.
Wider implications of the findings: After a failed cycle, patients should have the opportunity to integrate the new information into their decision-making. This would allow them to make a decision about continuing treatment with the doctor based on prognosis. Patients should also be offered additional decisional support to consider wider psychosocial implications of continuing treatment.
Trial registration number: N/A.

P-537 Relationship stability among heterosexual couples following oocyte and sperm donation

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Study question: Is there a difference in the relationship stability in couples having children via donated sperm or oocytes?
Summary answer: The results will be focusing on relationship dimension and also separation and custody discussions.
What is known already: Most research on couple's relationship stability following gamete donation has a short term perspective and measures relationship during treatment or shortly after treatment. The conclusions from these studies have been that the relationship stability has been found good in couples conceiving with different assisted reproduction techniques. The sample sizes are rather small in most studies.
Study design, size, duration: The present study is part of the "Swedish study on gamete donation" including all 7 clinics performing gamete donation in Sweden. All couples were consecutive recruited during 2005–2008. The couples have answered questions about their relationship three times before – at treatment and when the child was 1 and 4 years old. When the child was 7 years the men and women were again approached with question s about their relationship.
Participants/materials, setting, methods: The present study is part of the prospective longitudinal "Swedish study on gamete donation" including all fertility clinics performing gamete donation in Sweden. Parents with 7-year-old children following oocyte ($n = 53$) or sperm donation ($n = 79$) representing 75 families answered study specific questions about separations and custody. Among these families about one in six couples had separated (ORec 17%; SRec 18%).
Main results and the role of chance: For those who have been separated we found that in all but one family the parents had joint custody with the child living alternate weeks with its parents. The both the parents reported the custody situation to be functioning well. In one family following oocyte donation the mother had sole custody following a custody battle. We did not found any differences in separation frequencies in the couples.
Limitations, reasons for caution: The drop-out rate is of concern for the interpretation of the results. 45% for the SD-couples and 41% for the OD couples.
Wider implications of the findings: The couple's relationship is of interest to discuss at the treatment start and follow up.
Trial registration number: NA.

P-538 Which is the best instrument? A psychometric comparison of COMPI fertility problem stress (COMPI-FPSS), fertility problem inventory (FPI), and fertility quality of life tool (FertiQoL)

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Study question: Which infertility-related psychosocial adjustment self-report measure presents the best psychometric properties and better predicts clinically significant depression and anxiety in infertility patients?
Summary answer: While the COMPI-FPSS and the FPI presented better reliability, the FertiQoL presented better fit and discrimination of clinically relevant depression and anxiety.
What is known already: Infertility brings physical, emotional, and psychosocial challenges. About 40% of patients undergoing fertility treatments are at risk for psychological maladjustment. Several infertility-related psychosocial adjustment self-report instruments have been developed in the last decades. The most used have been the COMPI-FPSS, the FPI and the FertiQoL. This is the first study concurrently comparing the psychometric properties of these instruments and investigating their predictive validity to discriminate between patients with and without clinically relevant depression and anxiety in the same sample of patients.
Study design, size, duration: From November 2010 through July 2012, 1302 patients attending three Danish fertility clinics for fertility treatments were invited to participate. 919 patients declined to participate or did not respond (response rate = 29.4%). Ninety were further excluded because they did not meet the study's criteria. At one single moment, participants completed a questionnaire including sociodemographic questions and self-report instruments: COMPI-FPSS, FPI, FertiQoL, Beck Depression Inventory (BDI) and State-Trait Anxiety (STAI-State).
Participants/materials, setting, methods: Participants were 293 patients (161 women, 132 men). On average, participants were trying to conceive for 2.52 years (SD = 1.20) and 52.2% had already undergone one cycle of IVF/ICSI. Reliability, convergent and concurrent validity were evaluated. Confirmatory factor analyses (CFA) were performed for the three instruments. A receiver–operator curve (ROC) analysis was performed to evaluate the ability of each instrument to identify patients with or without clinically relevant depression (BDI >13) and anxiety (STAI-State ≥40).
Main results and the role of chance: The removal of a few items was performed after findings suggesting either an increase in internal consistency after removal (FPI and FertiQoL), or poor CFA model fit (loadings <0.40, FPI and FertiQoL). Subsequent analyses revealed good internal consistency in all three scales total scores (alphas between 0.89 and 0.91), with the COMPI-FPSS and the FPI subscales presenting better alphas than FertiQoL subscales. CFAs revealed acceptable fit for all final instrument models, with FertiQoL presenting a slightly better fit than the other two measures. Convergent validity was confirmed (r between 0.76 and 0.83). FPI results showed better concurrent validity indices than COMPI-FPSS and FertiQoL. In predicting clinically relevant depression, the areas under curve (AUC) were: COMPI-FPSS 0.86 (95%CI: 0.81–0.90), FPI 0.84 (95%CI: 0.79–0.89) and FertiQoL 0.88 (95%CI: 0.84–0.92). The AUC predicting clinically significant anxiety were: COMPI-FPSS 0.76 (95%CI: 0.70–0.81), FPI 0.77 (95%CI: 0.71–0.83) and FertiQoL 0.83 (95%CI: 0.78–0.88). The FertiQoL was the best instrument discriminating between patients with or without clinically relevant depression and anxiety and was able to

correctly discriminate 88% of the patients regarding clinical depression (sensitivity: 0.79; specificity: 0.81) and 83% regarding clinical anxiety (sensitivity: 0.79; specificity: 0.73) while COMPI-FPSS and FPI correctly discriminated lower percentages of patients.
Limitations, reasons for caution: These results cannot be generalized to infertile individuals not seeking treatment or without diagnosis. Because Cronbach's alpha is dependent on the number of items, the between-instrument comparability of reliability could have been affected. Further studies with repeated assessments are needed in order to evaluate responsiveness indices.
Wider implications of the findings: This study contributes to a better knowledge on psychometric properties of infertility-related psychosocial adjustment measures. This knowledge may help clinicians to decide which instrument to use in order to timely identify and refer to counseling or psychotherapy the patients who presented a greater vulnerability to develop maladjustment and emotional problems.
Trial registration number: NA.

P-539 Effect of IVF therapy on quality of sexual life

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Study question: Does IVF treatment affect sexual life of treated couple? What factors lead to lower quality of sexual life or its absence?
Summary answer: By 62% of respondents occurred decline of quality of sexual life. Main alteration was refusal of intercourse due to fear of negative therapy outcome.
What is known already: From psychological point of view infertility and its treatment are considered as very difficult process mostly unattended by any sexual dysfunction. The lack of sexual activity during treatment mainly by repetitive IVF cycles and fear of affecting the treatment outcome leads to emotional instability, relationship problems, sadness, anxiety, sexual alienation, etc. These issues progress at 20% of women into significant levels of depressive symptoms. Even if the routine psychological care is provided during fertility treatment, the fertility specialists do not deal with contentment in sexual life.
Study design, size, duration: The study was a retrospective questionnaire. Study duration was 7 months during 2015. There were addressed 450 participants among treated couples from the Czech Republic, Italy, Germany, Ireland and UK. The return in total was 127 (28%) filled questionnaires (84 women and 43 men).
Participants/materials, setting, methods: The participants were women and men undergoing a fresh IVF treatment or frozen embryo transfer. There was no difference between donor cycles and cycles with own oocytes and/or semen. The inclusion time was the day of HCG check-up (14 days after embryo transfer). There was no age limit by the participants; the average age was 39.5 years.
Main results and the role of chance: In 62% (79) of cases the respondents noticed deterioration of quality of their sexual life while undergoing IVF treatment, 37% (47) didn't feel any changes and 1% didn't answer the question. The most described alteration were: decrease in frequency of intimate activity 46%, decrease of sexual desire 20%, timing of intercourse only on fertile days 16%, change of sexual position 4%, change in use of erotic toys 2% and become unattractive to each other 2%. In 69% (58) of cases women didn't have intercourse after embryo transfer (ET). Reason for refusal was fear of negative result of the therapy 66%, fear of miscarriage 47%, barriers due to use of vaginal tablets 43%, tiredness 24%, pain in the lower abdomen 22%, dryness of vagina 10%. Also men didn't have intercourse after ET in the most cases (67%). In comparison to those results, 58% of women and 64% of men provided the same sexual desire as in the time with no treatment. Even 86% of women and 67% of men presented partner's interest in sex. In 34% of women and 56% of men the intercourse was compensated by non-coital activities (even with orgasm – 55%).
Limitations, reasons for caution: Main limitation was low return of completed forms and/or their general refusal due to non-anonymous sort of questionnaires. Non-anonymous processing ensured connection to patient's medical records for further analysis. Another limitation consisted in very intimate character of the inquiries and persisting general perception of the topic as a taboo.
Wider implications of the findings: Lack/absence of sexual activities, their lowered quality and fear of the impact on treatment result could negatively influence mental condition of treated patient. The situation could be worsened by repetitious IVF cycles after negative outcome. The treated couple should be openly informed about all options in sexual activities during therapy.
Trial registration number: –

P-540 "Just an egg"?: egg donors' perceptions and future expectations of being an identity-release donor

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Study question: How do UK egg donors think and feel about being an identity-release donor?
Summary answer: The conceptualisation of egg donation as "just an egg" and a recipients' chance for an "own child" influenced donors' views on anonymity, and information provision.
What is known already: Very little is known about how egg donors who have donated under an identity-release system think about their donation and future information exchange. The majority of identity-release egg donors surveyed 5–8 years after donating in a Swedish study were positive towards being contacted by offspring when they reached the age of eighteen (Isakkson et al., 2014). This is the first study of UK egg donors since the removal of donor anonymity in 2005.
Study design, size, duration: The findings were collected as part of an exploratory, in-depth qualitative study comprising of semi-structured interviews with eleven women who had attended an egg donation screening appointment at a UK clinic during a 4-month period in 2014.
Participants/materials, setting, methods: Interviews were conducted 2–6 weeks after the woman had donated or had withdrawn/been rejected from the donation process. Interviews explored egg donors motivations, experiences and future expectations of donating their eggs. Interviews were transcribed and thematic analysis performed.
Main results and the role of chance: The majority (91%) of egg donors conceptualised their donation as "just an egg," giving the recipient the chance to have an "own child." All egg donors were happy to be contacted by a child conceived through their donation, stating it was their "responsibility" to answer any questions they may have. However, the majority of participants thought contact unlikely: the child would either not be aware of their donor conception or would be comfortable with this information. In fact 45% of participants were unsure whether the removal of donor anonymity had been necessary, 36% had concerns about being an identity-release donor and only 36% provided non-identifying information about themselves at the time of donation in the form of a pen portrait and goodwill message. Concerns stemmed from empathy for the egg recipient and the possibility for donor information to make the donor's role more than "just an egg," intruding upon the recipient's "own child." All egg donors wanted information on the outcome of their donation and 64% wanted more information about the recipient(s) of their eggs in order to help them imagine the family they had helped to build through their donation.
Limitations, reasons for caution: This in-depth, exploratory study is the first study of UK egg donors since the removal of donor anonymity. However, due to its small sample size and recruitment from only one clinic, this study may not be representative of other egg donors.
Wider implications of the findings: This study highlighted the complexity egg donors experience in navigating information exchange. Further guidance should be given to egg donors when providing non-identifying information. Donors may feel more satisfied with their role as an identity-release donor if they receive more information about the outcome and recipient(s) of their donation.
Trial registration number: N/A.

P-541 The impact of disclosure versus non-disclosure on the child's well-being and on family functioning in heterosexual families with donor-conceived children: a systematic review

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Study question: What is the impact of (non)disclosure about donor conception on a child's psychological well-being and on family functioning in heterosexual families?
Summary answer: Overall, gamete donation families showed adequate marital, psychological and family functioning, though there are many gaps in knowledge as well as methodological shortcomings.
What is known already: The practice of gamete donation has in the past been surrounded by secrecy and concerns regarding the impact of these procedures on family functioning and the child's psychological well-being. Recent years has seen a gradual move towards more openness in society. Nevertheless, the impact of openness or secrecy on the child's socio-emotional development and family functioning remains still largely unknown.
Study design, size, duration: A systematic review was performed by searching electronic databases (PUBMED, PsycINFO, and Embase) by means of a specific search strategy followed by a snowball strategy. English language publications were screened for eligibility and subjected to a quality control using 22 criteria (STROBE). Relevant data were extracted to a structured data extraction sheet with different categories.
Participants/materials, setting, methods: Inclusion criteria: (i) only English language original empirical research with a quantitative, qualitative or mixed methodology, (ii) study participants were heterosexual couples who had used donor conception (sperm, egg or embryo) to conceive with donors who were either anonymous (unknown to recipients) or non-anonymous (known to recipients). (iii) the examination of (non)disclosure about a child's donor origin and its associated effects one of the primary aims of the study.
Main results and the role of chance: The initial search retrieved 1088 studies, dating from 1995 until 2012, of which 21 were retained for data extraction, conducted in 12 different countries, representing a diversity of disclosure customs about the child's donor origin. The majority of studies (N = 14) had a cross-sectional design whereas seven were longitudinal. Most studies used a mixed method approach, combining questionnaires with qualitative analysis (16/21). Sample sizes ranged from 7 families to 541 families, with a mean sample size of 141. An average of 14% (90/639) of DI couples had informed their child about their donor origin with an average age at disclosure of seven (SD = 3.8), as calculated over five studies (5/21), whereas 64% (409/639) of couples were non-disclosing. On the whole, the central finding of the review synthesis revealed no discrepancies between disclosing and non-disclosing families for marital relationship, child's and parents' psychological well-being, and family functioning. However, in disclosing families (15/21) both neutral as well as negative and positive reactions to disclosure were found, which appeared to be associated with age at disclosure. Concerning non-disclosure, some studies (3/21) noted that offspring showed an awareness of secrets within their families or raised questions regarding their family origins.
Limitations, reasons for caution: This review revealed several important methodological shortcomings in terms of sample size, data population and study design that may have biased the data in the studies. Therefore, data interpretation needs to take these shortcomings into account as well as several gaps of knowledge.
Wider implications of the findings: Our findings offer useful information to policy makers, professionals and prospective parents on the effects of (non) disclosure in gamete donation families. A framework of "selective disclosure" is proposed that goes beyond dichotomous thinking about (non)disclosure and focuses on the "good intentions" of parents regardless of their disclosure status.
Trial registration number: NA.

P-542 Reproduction by sperm donation and children information: a 10 years retrospective study of parent's disclosure in one French CECOS center

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Study question: To evaluate the percentage of parents that disclose their use of donated spermatozoa to their children, to know if parents disclosure is consistent with their intention before assisted reproductive techniques.
Summary answer: With a participation rate of 68%, most parents had already disclosed their use of donated spermatozoa to their children or intended to disclose it soon.
What is known already: In France, the sperm donation practice is supervised by CECOS (Centre d'Etude et de Conservation des 'ufs et du Sperme) centers. Each year, more than 2,000 couples have an ART using donated spermatozoa, and more than 50,000 children are born since the creation of the first CECOS in 1973. It seems that number of parents who intend to disclose their use of donated spermatozoa to their children regularly increase, but we have no data about the real parents attitude.
Study design, size, duration: In a retrospective study, we identified 486 pregnancies and 317 births resulting from ART with donated spermatozoa from 2002 to 2012. All couples gave their informed consent to be contacted after childbirth. We first contacted 164 couples by phone to explain the study objectives, 157 agreed to participate. We sent 157 questionnaires by mail to ask couples about their attitude and intention to inform or not their child.
Participants/materials, setting, methods: Families were recruited through our CECOS center. Participants completed a standardized questionnaire between February and March 2014.
Main results and the role of chance: Among 157 questionnaires sent, 105 couples answered and had 144 children born between 2002 and 2014. There were 41 couples that had already disclosed the donor origin to their child, and 64 (61%) who did not. Of the 41 couples (39%) who disclosed the donor origin to their child, 39 intended to before ART, but 2 (5%) did not want to before ART. Among the 64 couples (61%) who did not inform their child, 42 (66%) plan to inform their child soon, but 20 (31%) want to keep the sperm origin secret. Of the 20 couples who want to keep the origin secret, 3 intended to disclose the donor origin before ART.
Limitations, reasons for caution: This is a retrospective study. Although a good participation rate, the lack of response from 52 couples may induce a risk of selection bias.
Wider implications of the findings: This study shows that after childbirth the majority of parents disclose or intend to disclose the donor origin to their child, which is higher than reported in the international literature. Moreover, they are consistent with their intention before ART even if 5% of couples changed their intention after childbirth.
Trial registration number: No clinical trial.

P-543 Factors risk of infertility: what information patients receive before ART?

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Study question: What information is known couples before their treatment in ART and how they receive this information.
Summary answer: Many couples followed in ART are overexposed to infertility risk factors (smoking, overweight, alcohol, …) and often have a lack of information on the subject.
What is known already: In our modern society, changes in lifestyle increase exposure to certain factors affecting fertility and contribute to fertility decline (smoking, overweight, unhealthy diet, alcohol, …). The correction of these risk factors may allow patients to recover their potential natural fertility and improve outcomes in Assisted Reproductive Technologies (ART). When couples consult with ART, they have often not started to change these risk factors. At present where many means of communication are developed, couples can be informed but the target is not often achieved.
Study design, size, duration: We performed a descriptive epidemiological study, quantitative, with a self anonymous questionnaires offered to infertile couples consultant at the center AMP Saint-Roch in Montpellier on paper and the members of certain patient groups across the French territory by a questionnaire online from December to April, 2015.
Participants/materials, setting, methods: Three hundred and seventeen questionnaires women and 153 men were included. We asked in the first part of the questionnaire if they were exposed to various toxic or risk factors that may be involved in fertility. And in a second part of the questionnaire, the couples met on their knowledge and the means of communication that allowed them to be aware of these risk factors for infertility.
Main results and the role of chance: The average age of women is 34.5 years and men 36.3 years. The social level of patients is quite high with over 67% with a higher level of education. Most couples (75%) were already in ART: 75%

of patients accumulate between two and five risk factors. Addictions (tobacco, cannabis, alcohol) and stress are most evident for patients. Weight, diet and sedentary, although very common in our population are insufficiently known patients, especially men who are often the most exposed. Other factors (caffeine, toxic, testicular heat) are unknown to patients, but less frequent. Women seem better informed than men. The main source of information is the media and internet to more than one third of couples. The doctors only come in third position (15% by the gynecologist and 8.5% by the general practitioner) behind friends and family.

Limitations, reasons for caution: The survey by self-administered questionnaire can result in selection bias because they are the most involved patients who respond to the questionnaire. Patients already being in an IVF course, the information received may be considered retrospectively. Finally some questionnaires were incomplete.

Wider implications of the findings: The couples consulting for a problem of infertility, appear overexposed to risk factors and there is a lack of information for many factors. The sedentary lifestyle and stress are the main. Websites are the preferred means of patients, should be strengthened reliably, accurate and accessible.

Trial registration number: None.

P-544 Children's, parents' and donors' perspectives on family communication about sister-to-sister oocyte donation: a multi family member interview study

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Study question: How do family members (parents, children and the donor) experience family communication about the donor conception in sister-to-sister oocyte donation families?

Summary answer: Family communication about the donor conception was subject of careful consideration for both recipients and donors and was rather limited.

What is known already: In intra-familial medically assisted reproduction, familial closeness between donor and recipients raises questions about how family relationships are shaped and experienced before, during and after the donation. Studies on the recipient couples' experiences mainly focus on their disclosure decisions and the relationship between recipient couples and donors. The ways in which this donor conception is discussed and family members' individual experiences of the disclosure process do not receive much research attention.

Study design, size, duration: As part of a larger qualitative research project on family members' perspectives on social and genetic parenthood, semi-structured interviews were conducted with heterosexual couples, their oocyte donors and one of their children. Participants were recruited via the Department of Reproductive Medicine of the Ghent University Hospital.

Participants/materials, setting, methods: Couples eligible for the study were contacted by their counsellor 7–10 years post treatment. Two couples, one mother, three oocyte donors and three children were interviewed separately. Interviews were analysed using Interpretative Phenomenological Analysis, followed by an analysis within families and a comparison across families.

Main results and the role of chance: The three participating families all had disclosed the nature of conception to their (oldest) child. The parents held the belief that the way they spoke about the donation would impact their child's evaluation of this conception method. In this respect, carefully selected messages were given to the child and this was also what the child remembered of the story when they were asked about it in the child interview. Furthermore, disclosure processes in the recipients' and donors' families seemed to be coordinated as both the recipients and the donor wanted to avoid the children finding out in an inappropriate way. In general, there was only limited discussion about the oocyte donation between all family members. It is striking that some children expressed a wish to talk about the oocyte donation to the donor or the donor's children but refrained from doing so.

Limitations, reasons for caution: Our analysis was based on a small sample and does not intend to produce generalizable findings. Moreover, it was based on a specific subset of families in which the donor conception was disclosed to the child and parents felt comfortable with their child being interviewed.

Wider implications of the findings: This is found to be the first study linking children's, donors' and recipients' perspectives on family communication about the intra-familial donor conception. Consistent with recent process-oriented disclosure studies, this study pointed at the complexity of this dialogical process and to various meanings that can be attached to the donor conception.

Trial registration number: N/A.

P-545 Fertility preservation to transmen: learning the needs of a new patient group

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Study question: How does healthcare professionals at an academic fertility clinic experience transmen as a new patient group and adapt existing clinical fertility preservation (FP) program?

Summary answer: Learning the needs of transmen, a new patient group, was a challenging process. Through increased knowledge, training and patient collaboration, professionalism grew over time.

What is known already: Earlier research shows that FP may negatively impact transmen's gender dysphoria, as the procedures are closely linked to the female identified body parts the men strive to leave behind. Trans people have reported negative experiences of healthcare encounters, both within general health care as well as in reproductive care, where they have experienced discrimination and even been refused care. No studies have investigated healthcare professionals' experiences of caring for transmen who undergo FP. Research among general healthcare providers' shows that the professionals felt that they lacked knowledge about trans-specific healthcare needs and available resources.

Study design, size, duration: Healthcare providers working at an academic reproductive medicine clinic providing FP for transmen were invited to participate in semi-structured interviews. As we wanted to capture the experiences of meeting transmen throughout the chain of care, we also included administrative staff. The interviews lasted 17–53 min (mean 34 min). The interviews were digitally recorded and transcribed verbatim. Inclusion started in January 2016 and is still ongoing.

Participants/materials, setting, methods: Hitherto, 10 health care providers (2 physicians, 4 midwifes and 4 administrative personnel) have participated in the study. The interviews covered two areas: the preparation, education and planning for this new patient group which took place prior to the new patient group being counselled for FP, and personal experiences of caring for transmen that underwent counselling and FP. Data was analysed using thematic content analysis.

Main results and the role of chance: Preliminary analysis resulted in three themes: *preparations*, *encounters* and *practicalities*. The first theme is about how the clinics preparations, such as presentations and meetings, helped the health care providers to understand the transmen's specific needs. The second theme is about experiences of meeting the transmen who underwent FP procedures. The most challenging part in the encounters was how to know what name, pronoun and which terminology to use. Fear to upset or irritate lead to holding back on communication. One way to solve this was to ask about preferred pronouns and words and being responsive to any signals. The healthcare providers noted that they became more skilled over time. The physicians and midwifes were particularly cautious when performing pelvic exams, as they were considered to be distressing. The third theme is about practicalities that sometimes functioned as a barrier to optimal care, such as the printed patient information material not being adjusted for the new patient group. Additionally, IT system issues when recording new personal identity number following the patients changing their legal sex were encountered. These problems were all solved over time, but caused distress not only for the transmen but also for the health care providers.

Limitations, reasons for caution: As in all qualitative research the results are based on an interpretation of the interviews. So far, only ten providers have been interviewed and some caution is advised when interpreting the results.

Wider implications of the findings: The findings of the study provide an insight into what facilitators and barriers that may be present when introducing a new patient-group at a reproduction clinic; transmen undergoing FP. This in

turn may help other clinics to prepare for the patient group in order to optimize the care.
Trial registration number: Not applicable.

P-546 Does having a child impact infertile couples' relationship satisfaction? A longitudinal study with men and women who did and did not achieve a live birth

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Study question: Does having a child impact infertility patients relationship satisfaction?
Summary answer: Having a live birth seems to increase relationship satisfaction 5 years after a couple seeks treatment.
What is known already: Literature suggests that the infertility process (i.e., infertility diagnosis, fertility treatments) and the inherent psychological phenomena (e.g., common goal, complicity) can bring marital benefit to infertile couples. However, some scholars argue that such marital benefit may fade with time and/or discontinuation of treatments. Furthermore, transitioning to parenthood has a negative impact on couple's relationship satisfaction. The challenges that accompany taking care of a newborn seem to push the members of the couple away from each other. There is no long-term evidence on how this transition affects infertility patients.
Study design, size, duration: Participants were drawn from a consecutive sample of patients attending a fertility center in 2010/2011 (T1), who were contacted via telephone in 2015 (T2). Each woman was asked to report current relationship status, fertility treatments outcome, reproductive decision, and relationship satisfaction. If the couple was not divorced/separated, men were then contacted to report his relationship satisfaction as well.
Participants/materials, setting, methods: Exclusion criteria were being divorced or separated, having previous children and having adopted. The final sample was composed of 107 couples (81 had achieved a live birth, 26 had decided on a child-free lifestyle). Patients responded to the satisfaction subscale of the *Perceived Relationship Quality Components Inventory* at both assessment points. Reproductive decisions (T2) were assessed via a brief semi-structured interview. Paired-sample *t*-tests were performed for males and females separately.
Main results and the role of chance: No baseline differences were found on relationship satisfaction between couples who would and would not achieve a live birth after treatments ($p > 0.05$). No differences between satisfaction in T1 and T2 were found for men ($M_{T1} = 5.48$; $SD_{T1} = M_{T2} = 5.57$; $SD_{T2} =$) or women ($M_{T1} = 5.51$; $SD_{T1} = 0.688$; $M_{T2} = 5.67$; $SD_{T2} = 0.688$; $p > 0.05$) who decided on a child-free lifestyle. For those who had a live birth between the two assessments, an increase in relationship satisfaction was found both for men ($M_{T1} = 5.34$; $SD_{T1} = 0.667$; $M_{T2} = 5.64$; $SD_{T2} = 0.676$; $p = 0.005$) and women ($M_{T1} = 5.33$; $SD_{T1} = 0.778$; $M_{T2} = 5.57$; $SD_{T2} = 0.680$; $p < 0.001$).
Limitations, reasons for caution: Although this study extended the evaluation of relationship satisfaction almost to 5 years after having started treatment, we still do not know whether marital benefit persists over a longer period. Findings cannot be generalized to patients who transitioned to parenthood through adoption.
Wider implications of the findings: The increased marital satisfaction that infertile patients experience from transitioning to parenthood is encouraging, and fertility staff can use this information in preventing treatment discontinuation. Further studies are needed to elucidate if and how infertile couples enjoy infertility marital benefit by assessing pre-treatment levels.
Trial registration number: N/A.

P-547 Surrogate families in Spain: difficulties perceived of doing the process abroad, perception and relationship with the surrogate and decision over disclosure

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Study question: Psychosocial experience of surrogacy in Spanish families who have done the process aboard.
Summary answer: Spanish surrogate families also have to face issues related with living the distance and the lack of proximity with the surrogate mother.
What is known already: No studies have been done about surrogacy in Spanish population. Due to the illegality of the process in Spain, as in many other European countries, families have to look for a commercial surrogacy that implicates emotional, economic, cultural, social and family issues.

Findings in other countries, where national surrogacy is allowed, suggest that surrogate families maintain good relationships with the surrogate mother over time, report a positive arrangements experience and show disclosure intention.
Study design, size, duration: Data were collected from January to November 2015 in a cross sectional study in Spain. We enrolled 35 men and women, couples and single, who had done a surrogacy process aboard.
Participants/materials, setting, methods: The sample is composed by heterosexual and homosexual couples, as well as single gay men and women in Spain, who had at least one surrogacy child older than 6 month.

Semi-structured, open-ended and auto administrated questionnaire relating to motivations, experiences, relationship with de surrogate mother, support, knowledge, information, concerns, legality, genetic link and disclosure, were sent by e-mail to the families, previous telephone contact.

Participant where recruited through surrogacy agencies and intended parents national associations.
Main results and the role of chance: Results show 46.7% of the sample would modify some aspect of the surrogate process, especially those related to legal (16.7%) and administrative aspects, registers and information (16.7%). Parents describe positive aspects of surrogacy as (1? y 2?) (29.9%), and the negative ones are related to legal aspects (43.5%), distance, lack of information and the economic cost (13.5%). The main difficulties these families have had to face are economic (66.5%), job (26.6%) and language (26.6%). Having done the surrogation in their own country would have allow them to have much more implication during the process (53.2%) and establish a closer relationship with de surrogate mother (49.9%). After meeting more than one possible surrogate mother (80%) intended parents established a close relationship with her, describing her as nice, affective or self confident. Contact between the intended parents and the surrogate mother decreased over time. 13.8% of the parents have informed their children about surrogacy and 82.7% of them show disclosure intention as their children get older. Narrative about the surrogacy process includes, in 51.7% of the cases information and data about the process and 34.5% of them manifest gratitude toward the surrogate mother. 58.6% of the surrogate children have met their surrogate mother.
Limitations, reasons for caution: Limitations: the sample size is relatively small due to the difficulty to identify those families and the legal situation they face. Confounders: infertility problems, genetic link, marital status, gender, motivations, sexual orientation, economic status.
Wider implications of the findings: Distance is the most important surrogacy stressor, generating anxiety, fear, lack of control and economic issues. Results are consistent with the literature, in the perception of the surrogate mother and the disclosure intention. Data show great ignorance about how and when disclosure. Contact with the surrogate mother decreased over time.
Trial registration number: x.

P-548 Can pre-treatment emotional health predict pregnancy rates after IVF?

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Study question: Can pre-treatment emotional health predict pregnancy rates after *in vitro* fertilization (IVF) treatment?
Summary answer: Pre-treatment emotional health does not predict pregnancy rates after IVF.

What is known already: The emotional burden of infertility is increasingly becoming a significant priority. Studies on psychological interventions suggest a link between emotional health improvement and IVF outcome. The evidence from existing literature demonstrates higher levels of anxiety and depression in IVF patients, but the effect this has on the conception rates is inconclusive. Existing studies have methodological limitations due to use of generic tools to assess emotional health which cannot capture the complex nature of fertility specific distress. There is a need for large cohort study to examine this relationship using a fertility specific tool.
Study design, size, duration: The aim of this study is to investigate if emotional health of patients can be used to predict IVF outcome. The study was designed by recruiting a prospective cohort of 414 women undergoing IVF between June 2011 till February 2015. The treatment cycle of 35 women were cancelled at different stages due to medical reasons. 379 patients completed the treatment cycle. An intention to treat analysis was performed.
Participants/materials, setting, methods: Women undergoing IVF were asked to complete a set of patient-reported outcomes at the start of the cycle which included Emotional Health in Infertility Questionnaire (EHIQ), Perceived Stress Scale, Positive and Negative Affect and Fertility Problem Inventory. Clinical pregnancy rates and live birth rates were compared between women with low, average and high emotional health. We then used logistic regression to see if pre-treatment emotional health can predict the probability of pregnancy.
Main results and the role of chance: 414 women undergoing IVF were divided into three tertiles according to their emotional health profile: poor emotional health (EHIQ 13.13–56.88; n = 140), average emotional health (EHIQ 57.5–71.88; n = 139) and high emotional health (72.5–97.50; n = 135). The three groups were similar with regards to female age, BMI, previous live births and proportion of patients having their first IVF. The overall pregnancy rate is this study group was 31.4%. Clinical pregnancy in patients with low emotional health was statistically similar to patients with high emotional health (27.9 vs. 31.1% p = 0.597). The clinical pregnancy rate was lowest (27.9%) in women with poor emotional health, but the highest pregnancy rates were found in women with average emotional health (36.2%). This difference was not statistically significant (27.9 vs. 36.2% p = 0.199). Logistic regression showed that EHIQ scores did not predict the clinical pregnancy in this study group.
Limitations, reasons for caution: Some patients who declined to participate, cited stress about the treatment outcome as the main reason. This indicates selection bias as some patients with poor emotional health have not been included.
Wider implications of the findings: Our study has helped to identify a group of patients with poor emotional health at the start of IVF, who are at risk of significant psychological distress following a failed cycle. Psychological interventions may be indicated in this group and help reduce dropout rates.
Trial registration number: Not a randomised control trial. Regional Ethics Committee reference number: 10/H1308/46.

POSTER VIEWING SESSION

QUALITY AND SAFETY OF ART THERAPIES

P-549 A comparison of dual triggering by administration of GnRH agonist plus HCG versus HCG in normal responders in ART outcomes

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Study question: Question: does the mode of triggering final oocyte maturation influence the ART (artificial reproductive technology) outcome in normal responders?
Summary answer: It seems that we can improve the ART outcome by adding GnRH agonist to standard dose of hCG in normal responders.
What is known already: Several studies in high responders showed significant improvement in IVF outcomes when a dual trigger was used without a significant increase in the OHSS rate. As we know that GnRH-a causes both FSH and LH release like a physiologic natural cycle and according to the fact that gene expression pattern and downstream signaling of LH receptors is different between hCG and GnRH-a triggered patients, several investigators intended to study the effect of coadministration of GnRH-a and hCG triggering to improve ART outcomes.
Study design, size, duration: This double-blind randomized controlled trial was performed at the Research and Clinical Center for Infertility, Shahid Sadoughi University of medical Sciences between April 2014 until February 2015. A total of 223 patients were assessed for eligibility.
Participants/materials, setting, methods: The inclusion criteria were tubal or male infertility, BMI < 32, age ≤42 years, absence of major endocrinologal pathology, first, second and third IVF cycle.

All patients began ovarian stimulation with flexible antagonist protocol. When at least two leading follicles had reached 17 mm in diameter, final oocyte maturation was triggered by either 6500 IU hCG alone, or by 6500 IU hCG plus 0.2 mg of triptorelin.
Main results and the role of chance: A total of 184 cycles were analysed. We found no statistically significant differences in the total recombinant FSH dose, duration of stimulation, duration of GnRH-antagonist treatment, serum E2 and on the day of trigger. Although mean number of oocytes retrieved and mature metaphase II (MII) oocytes and obtained embryos were higher in the dual-trigger group compared with the controls (10.85 versus 9.35 and 8.80 versus 7.98), the observed differences were short of reaching statistical significance (p-value: 0.009 and 0.12). Also the difference of OHSS rate between the two groups were not statistically significant (p-value = 0.06).

Our results was not significantly different except a trend toward higher oocyte and embryo yield and also clinical and ongoing pregnancy rates in dual trigger group (26.3 versus 22.6% and 24.2 versus 22.5) (p-value: 0.3 and 0.77) (p-value = 0.3).
Limitations, reasons for caution: We think that this new concept require more study before becoming a universal COH protocol in IVF practice. Certainly, extending the study population will help to get a better result.
Wider implications of the findings: The results of our study did not confirm the favorable effect of dual-triggered oocyte maturation with a GnRH-agonist and a standard dosage of hCG that was shown by Lin et al. (2013)[9] as an effective strategy to optimize pregnancy outcome for normal responders in GnRH-antagonist cycles.
Trial registration number: The study was registered under IRCT2015031221420N2.

P-550 The long-term alterations of myocardial remodeling associated genes from birth to old age in assisted reproductive technology mice and mechanism involved

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Study question: Could assisted reproductive technology (ART) have adverse effects on cardiovascular long-term health? Besides, what are likely mechanisms involved in these alterations?
Summary answer: The expression of myocardial remodeling associated genes are significantly up-regulated in offspring conceived by ART compared with the naturally conceived offspring.
What is known already: There is evidence to suggest that ART can increase the risk of cardiovascular birth defects and high blood pressure in offspring. However, the long-term effects of ART on cardiovascular system and molecular mechanisms remain unclear.
Study design, size, duration: C57BL/6J female and male mice were used in this study. They were randomly divided into *in vivo* group, IVF group and ICSI group. Institute of Cancer Research (ICR) female mice that were at least 8 weeks of age were used as pseudopregnant recipients to get their offspring. All treatment protocols involving the use of animals were approved by the Zhejiang University Animal Care Committee according to the Institutional Guidelines for Animal Experiments (No. ZJU2009101007Y).

Participants/materials, setting, methods: The body weight, blood pressure and heart rate were detected from birth to the age of 1.5 year in control group, IVF group and ICSI group. At the same time, the myocardium of mice offspring was respectively detected at 3 week, 10 week and 1.5 year. The expression of myocardial remodeling associated genes (COL1, COL3, CTGF, Myh7), AT1, AT2 and Akt were detected by real-time quantitative PCR, Western blot, immunofluorescence and pyrosequencing.
Main results and the role of chance: The results showed that the expression of COL3 and CTGF were significantly up-regulated both in myocardium of IVF group and ICSI group compared with control group ($P < 0.05$ and $P < 0.01$ respectively) at the age of 3 week. At the age of 10 week, the expression of COL1, COL3 and CTGF were significantly up-regulated in IVF group and ICSI group ($P < 0.01$, $P < 0.05$ and $P < 0.05$ respectively). When the mice reached the age of 1.5 year, the expression of COL1, COL3, CTGF and Myh7 were significantly up-regulated in IVF group and ICSI group compared with control group ($P < 0.01$, $P < 0.05$, $P < 0.05$ and $P < 0.05$ respectively). What is more, there was obvious different in the expression level of these genes between IVF group and ICSI group in old age ($P < 0.05$). Besides, DNA methylation rates of these genes were also different among three groups ($P < 0.05$). Furthermore, the AT1, AT2, Akt and p-Akt expression in myocardium were significantly different among three groups ($P < 0.05$) which had correlation with expression of myocardial remodeling associated genes.
Limitations, reasons for caution: The advantage of this study is that the long-term cardiovascular conditions can be observed in ART mice, especially in old age. Although we observed gene alterations in myocardium of ART mice compared with controls, further studies are urgently needed to confirm this observation and determine the cause of this phenomenon.
Wider implications of the findings: In this study, we report an association between ART and myocardium remodeling which could be a reflection of adverse effects of *in vitro* culture conditions. Meanwhile, ART offspring would be more vulnerable in cardiovascular diseases in the long term.
Trial registration number: This study is not clinical trial.

P-551 Neurodevelopmental and cardiometabolic outcome in 4-year-old twins and singletons born after *in vitro* fertilization

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Study question: Do cognitive development, neurological condition, anthropometrics and blood pressure (BP) of 4-year-old *in vitro* fertilization (IVF) twins differ from those in 4-year-old IVF singletons?
Summary answer: Four-year-old IVF twins had lower sequential intelligence quotient (IQ) and higher systolic blood pressure (SBP) than IVF singletons after adjustment for confounders and effect mediators.
What is known already: Twin pregnancies are associated with worse perinatal outcome than singleton pregnancies. This also holds true for IVF twin pregnancies: IVF twin pregnancies are associated with an increased risk of obstetrics complications, such as preterm birth, low birthweight, caesarean section and perinatal mortality. Despite the high number of multiple births in IVF pregnancies and the increased risk of obstetric complications in IVF twins, limited knowledge is available on the cognitive development, neurological condition, anthropometrics and cardiometabolic health of IVF twins compared with IVF singletons.
Study design, size, duration: A prospective assessor blinded follow-up study of two studies: the Groningen assisted reproductive technology (ART) cohort study and the Preimplantation genetic screening (PGS) Follow-up study. In total 151 children born following controlled ovarian hyperstimulation IVF (COH–IVF) were assessed. Overall attrition rate was less than 15%. Information on socioeconomic status, the prenatal, perinatal and neonatal period was collected two weeks after birth on standardized charts. The follow-up examination took place at the age of 4 years.
Participants/materials, setting, methods: Participants were 4-year-old singletons ($n = 103$) and twins ($n = 48$) born following COH–IVF as part of the Groningen ART cohort study and the PGS Follow-up study. Primary outcome was Total IQ, evaluated with the Kaufman Assessment Battery for Children, second edition. Secondary outcomes were neurological condition (evaluated with the Hempel assessment), anthropometrics and BP. After univariable analyses, multivariable regression analyses were used to adjust for effect modifiers, such as gestational age, and additional confounders.
Main results and the role of chance: Parental characteristics, fertility parameters and child characteristics were similar, but birth characteristics differed between twins and singletons. Twins had, for example, a lower birthweight ($p < 0.001$) and lower gestational age at birth ($p < 0.001$). Total IQ (twins [mean 94.96, SD 10.91] and singletons [mean 99.24, SD 11.86]), sequential IQ, simultaneous IQ, and knowledge IQ scores of twins were lower than those of singletons. Learning IQ scores of twins were similar to those of singletons. Also weight and height of twins were significantly lower than those of singletons (16.9 ± 2.1 vs. 18.6 ± 2.8 kg and 105.8 ± 3.4 vs. 108.7 ± 5.3 cm, resp.). But triceps and subscapular skinfold thickness in the two groups were similar. The neurological condition of twins did not differ from that of singletons. In the unadjusted analysis SBP (regression coefficient [B]: 0.53, 95% confidence interval [95% CI] [2.32 −3.40) and diastolic blood pressure (DBP) of twins were similar to those of singletons. Most differences between twins and singletons disappeared after adjustment for confounders. Yet, the multivariable regression analyses revealed that twins differed in two ways from singletons: twins had a lower sequential IQ, (B: −5.98, 95% CI [−11.27 to −0.68]) and they had a higher SBP (B: 4.67, 95% CI: 0.64–8.71), ($p = 0.024$) than singletons.
Limitations, reasons for caution: We did not distinguish between monozygotic and dizygotic twins. Monozygotic twins might contribute to the increased risk for unfavourable outcome of the twin group. However, the majority of IVF twins was probably dizygotic due to dual embryo transfer. Another limitation is that both BP measurements were performed on 1 day.
Wider implications of the findings: Our study suggests an IVF twin effect on short-term memory and SBP. It is conceivable that twin pregnancies are associated with increased levels of fetal stress, originating from maternal psychological stress and physiological stress related to less favourable uterine conditions for twins. IVF may add to the risk or stress.
Trial registration number: M09.074824.

P-552 Factors affecting birthweight after single frozen-thawed embryo transfer

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Study question: Does blastocyst grade affect birthweight after frozen-thawed embryo transfer (FET)?
Summary answer: Birthweight of the singleton born after FET was not associated with blastocyst morphology but related to maternal body mass index (BMI) and smoking habits.
What is known already: A previous study (Fertil Steril 2015 103: 382) demonstrated that greater birth weight was associated with a higher inner cell mass grade after standard *in vitro* fertilization (IVF) and FETs. However, it is still a controversial topic and no consensus on whether blastocyst grade affects fetal growth.
Study design, size, duration: A retrospective analysis was performed for 1145 singleton neonates born after FET cycles between April 2008 and March 2014.
Participants/materials, setting, methods: The examined factors affecting birth weights of singletons born full-term after FET cycles included quantitative variables like gestational age, cryopreservation period, maternal age, BMI, and endometrial thickness and qualitative variables like newborn gender, parity, ovarian stimulation and insemination methods, maternal smoking habits, embryonic stage, and blastocyst morphologic parameters. All blastocysts were cultured in same culture Media (Cleavage and Blastocyst Media Cook®). The blastocysts were graded according to the Gardner grading system.
Main results and the role of chance: A total of 1145 deliveries resulting from single blastocyst transfers were analyzed. A correlation analysis revealed that the quantitative variables including gestational age ($r = 0.399$, $P < 0.0001$) and BMI ($r = 0.177$, $P < 0.0001$) significantly correlated with birthweight. No

significant differences were observed for birthweight and ovarian stimulation and insemination methods. There was no significant difference in the average birthweight between the high grade blastocysts ($n = 923$: 3125.7 ± 385.9 g) and the low grade blastocysts ($n = 222$: 3102.4 ± 357.7 g). Stepwise logistic regression indicated gestational age, newborn gender, BMI and maternal smoking habits as effective factors to predict birth weight. After correction using these variables, a multivariate regression analysis resulted in significant *F*-statistic values for expansion ($F = 0.085$, $P = 0.968$), ICM ($F = 0.379$, $P = 0.685$), and TE ($F = 0.810$, $P = 0.445$). Therefore, blastocyst morphologic parameters were suggested to be ineffective factors to predict birth weight.

Limitations, reasons for caution: As this study was retrospective, blastocyst selection was based on existing selection criteria. We were not able to adjust the analyses for paternal BMI, maternal weight gain during pregnancy and quality of diet due to limited database information. Further studies are needed to clarify this issue.

Wider implications of the findings: Birthweight of low grade blastocyst is similar to that of high grade blastocyst. Our findings suggested that maternal factors affected the birthweight more than embryonic factors.

Trial registration number: Not applicable.

P-553 ART laboratory disinfectants are not "embryosafe" and should always be used with caution

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Study question: To evaluate the effects of six laboratory disinfectants simultaneously when used in close proximity to sperm and embryos using a variety of assays.

Summary answer: All six disinfectants demonstrate toxicity in at least one assay and all should be used with caution.

What is known already: As ethanol is a known volatile organic compound (VOC), non-VOC disinfectants have been promoted for use in ART labs, with varying ingredients and actions: Oosafe® (sparMED) and Fermicydal D2® (Labotect) contain quaternary ammonium compounds (QACs) whereas Fertisafe™and Fertisafe Plus™ (Research Instruments) are chlorine and silver dihydrogen citrate based respectively. 3% hydrogen peroxide (H_2O_2) is also used in ART laboratories. There has been no previous cytotoxic testing comparing all 6 disinfectants simultaneously.

Study design, size, duration: 1-cell mouse embryo and human sperm assays (*direct contact*: 1% v/v disinfectant in media, or *atmosphere:* incubators/chambers cleaned with disinfectants just prior to use, and *residue:* culture dish with dried-rinsed disinfectant residue (embryos only) were used to test all disinfectants against controls.

Participants/materials, setting, methods: Embryos were randomly distributed to each disinfectant scenario (3 replicates, $N = 528$). Development and survival was assessed daily to the blastocysts stage. Sperm (4 replicates) was centrifuged through a density gradient, diluted and divided between *direct contact* (0.5 ml, capped tubes), *atmosphere* (0.5 ml uncapped tubes in disinfectant cleaned, un-gassed chambers) and controls. Motility was assessed at 24, 48 and 72 h. Sperm motility loss was the difference of total motility at time 0 and the assessment time.

Main results and the role of chance: For *direct contact* and *residue*, lysis and/or no cleavage was observed with the H_2O_2, Oosafe, Fermicidal and Fertisafe Plus groups, whereas, limited blastocyst rates were observed in Ethanol [r*esidue*: 4/13 (31%), *direct contact* 2/31 (6.5%)] and Fertisafe [residue 1/20 (5%) and *direct contact* 1/16 (6.25%)], all except ethanol *residue* were significantly different to controls [$P < 0.05$, 35/59 (59.3%)]. For the embryo *atmosphere* trials, H_2O_2 and Fertisafe groups showed the lowest development to blastocysts (1/18 and 0/44 respectively, $P < 0.001$) while Fermicidal (17/32, 53%) was the only group not different from the controls [35/59 (59.3%)]. For *direct contact* there was significant sperm motility loss after 24 h ($P < 0.01$) in all treatment groups (>90% loss) compared to the control (<35% loss), and for *atmosphere,* significant sperm motility loss was only observed in the Ethanol, Fertisafe Plus and H_2O_2 groups ($P < 0.01$). Paired Chi square and Dunnets multiple comparison *post-hoc* tests were used on embryo development and sperm motility respectively.

Limitations, reasons for caution: Treatment conditions used are representative of worse-case scenarios thus, in ART laboratories all these disinfectants may be safe to use in some circumstances. Also, *atmosphere* conditions are hard to standardise: disinfectants were rotated through bench-top incubators (confined space but continual gas-flow) and traditional CO_2 incubators (large space, reduced gas movement).

Wider implications of the findings: Despite concern with Ethanol, its quick evaporation and apparent reduced toxicity implies that moderate use maybe acceptable. QACs in *atmosphere* had reduced toxicity, but lethal if any contact occurred. H_2O_2 proved lethal in all testing scenarios. This research leads to a greater awareness of disinfectant toxicity.

Trial registration number: Not applicable

P-554 Comparison of birthweights in patients randomly assigned to fresh or frozen-thawed embryo transfer

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Study question: Are fresh and frozen-thawed embryo transfers (FET) associated with different birthweight in resulting infants?

Summary answer: Birthweights following fresh transfer were significantly less than those following FET.

What is known already: Multiple prior studies of national registries have found significantly reduced birthweight in fresh transfer when compared to frozen-thawed embryo transfer. Reported differences in mean birthweight observed in registry studies have ranged from 40 to 250 g, with a median reported difference of 145 g. However, registry studies are inherently vulnerable to confounding effects of non-randomized treatment assignment and unknown/uncontrolled variation in treatment protocols.

Study design, size, duration: This follow-on study included 134 patients that had live birth after being randomly assigned to fresh transfer or FET. Pregnant subjects were followed through childbirth. No subject was lost to follow-up.

Participants/materials, setting, methods: All patients underwent ovarian stimulation with both hMG and rFSH at a private fertility center. Immediately following oocyte collection, patients were randomly assigned to either fresh blastocyst transfer or else cryopreservation (conventional slow freezing) of the entire cohort at the bipronuclear stage followed by thaw of the entire cohort and culture to the blastocyst stage before transfer. Identical culture conditions were used in both groups and all transferred embryos were primary (not supernumerary) blastocysts.

Main results and the role of chance: After adjusting for potential confounding variables, including gestational age at birth, number delivered, and presence of a vanished twin, the adjusted mean birthweight was 166 g less following fresh blastocyst transfer when compared to FET ($P = 0.0094$) (95% CI 42–291 g).

Limitations, reasons for caution: This data set is much smaller than those used in registry studies of birthweights. The specific causal mechanism (embryonic or uterine) cannot be discerned from this study design.

Wider implications of the findings: The birthweight differences between patients randomized to fresh transfer or FET are consistent with previous registry studies, suggesting the previous findings were not caused by potential confounding resulting from non-randomized patient allocation or uncontrolled protocol variation, including culture conditions, stage of transfer, and/or use of supernumerary embryos.

Trial registration number: ClinicalTrials.gov registration numbers NCT00963625 and NCT00963079.

P-555 Failure mode and effect analysis as a proactive method to prevent risks in assisted reproduction technology (ART): a more than 1000 cycle-laboratory experience

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Study question: Is failure mode and effects analysis (FMEA) a useful and effective method of risk assessment in the ART laboratory?

Summary answer: The analysis of the potential failures, their causes and effects has allowed to improve the safety strategies already adopted in the clinical practice.
What is known already: Over the years, there have been numerous reports of misidentification resulting at best in a deleted cycle if the mistake was identified before embryo transfer, or in a tragedy if realized after the embryo transfer. Therefore, approaches that systematically supervise the whole process and identify the causes of all errors are needed. FMEA represents a strategy to identify and eliminate known and/or potential failures before they occur.
Study design, size, duration: FMEA represents a proactive risk evaluation technique used to identify and eliminate known and/or potential failures. The project was conducted in a large ART center that performs more than 1000 "fresh" cycles and about 400 "frozen/thaw" cycles per year and it was applied between August 2013 and August 2014.
Participants/materials, setting, methods: Processes were analyzed to identify and score the potential failure modes, using the risk priority number (RPN) scoring system. The magnitude of the RPN, by multiplying 3 factors, occurrence, severity, detection (graded 1–5), denotes the priority of the failure mode. The final step of FMEA is to optimize the single procedure according to the RPN score. Finally, FMEA was repeated after 8 months and the improved RPN after the corrective actions was calculated.
Main results and the role of chance: In total, 11 individual steps in our ART laboratory were identified and mapped. All the steps had multiple failures and sixty-eight different potential failure modes were identified. The highest ranked failure modes with an RPN score of 25 encompassed 17 failures and pertained to "patient mismatch" and "biological sample mismatch". The maximum reduction in risk with RPN from 25 to 5 was mostly related to introduction of double checks by a second operator. The critical failure modes about sample processing have been improved by 50% in RPN focusing on the training in order to correct technical errors of the staff. Three indicators of FMEA success based on technical skill, competence and traceability have been evaluated after FMEA implementation. The monitoring of these three indicators represented a substantial proof that the corrective actions applied exerted a positive influence on daily work. Double witness by a second human operator should be introduced in the lab as the main control measure used to avoid sample mix-ups.
Limitations, reasons for caution: This study is subjected to the general limitations of the FMEA method, such as subjective experiences of the individual participants for which failures may be unrecognized ("missed"), underestimated or exaggerated. Other limitations were seen such as its time-consuming nature, the difficulty with the scores and its partial validity and reliability.
Wider implications of the findings: The goal of the method of risk assessment should be to completely eliminate risk of mismatching. FMEA is capable of producing a wealth of information about the potential vulnerabilities within the process and consequently provides systematic guidance for development of risk-reduction interventions.
Trial registration number: Not applicable.

P-556 Fresh versus frozen-thawed embryo transfers: a systematic review and meta-analysis of obstetric outcomes

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Study question: Are there differences in the risks of obstetric outcomes in IVF/ICSI pregnancies when comparing fresh to frozen- thawed embryo transfers (FET)?
Summary answer: The results of this systematic review and meta-analysis suggest that pregnancies from FET have an overall better obstetric outcomes when comparing to fresh embryo transfer.
What is known already: Although fresh embryo transfer is currently the norm in assisted reproductive therapies (ART), there are increasing concerns regarding the adverse effects of controlled ovarian stimulation (COS) upon the endometrial and uterine environment. In contrast, FET is performed in a more "physiological" environment and this could be related to better outcomes after FET when compared to fresh cycles. It has been suggested that ART pregnancies are related to poorer obstetric outcomes when compared to natural conceptions. However, the obstetric outcomes observed in pregnancies arising from ART may differ among fresh and FET cycles.
Study design, size, duration: We conducted a systematic review and meta-analysis of observational studies published in English, comparing obstetric outcomes in singleton pregnancies after fresh and FET. An electronic search was performed within the PubMed, EMBASE and Cochrane databases, through October 2015. We also searched the references of the relevant articles.
Participants/materials, setting, methods: Original studies reporting obstetric outcomes for singletons pregnancies after ART that compared fresh to FET outcomes were included. Studies including only frozen and donor oocytes were excluded. The main outcomes were ectopic pregnancy and preterm birth (delivery <37 weeks of gestation). The secondary outcomes were pregnancy-induced-hypertension (PIH), pre-eclampsia, placenta previa, placenta accreta, and placental abruption. Adjusted odds ratio (aOR) was used to calculate the estimated effect of outcomes, with the exception of ectopic pregnancy.
Main results and the role of chance: The search yielded 915 articles, 43 of these met inclusion criteria, reporting on obstetric outcomes of over 1,000,000 pregnancies. When comparing pregnancies arising from FET to fresh embryo transfer, there was a decrease in the risk of obstetric complications in pregnancies from FET when compared to fresh embryo transfer: ectopic pregnancies (all embryo developmental stages – including 929,431 pregnancies) – odds ratio (OR) 0.80, 95% confidence interval (CI) 0.67–0.95; ectopic pregnancies after cleavage stage – OR 0.73, 95% CI 0.65–0.81; ectopic pregnancies after blastocyst stage – OR 0.52, 95% CI 0.42–0.64; preterm birth (219,356 singletons pregnancies evaluated) – aOR 0.90, 95% CI 0.84–0.96); and placental abruption (59,450 singleton pregnancies evaluated) – aOR 0.65, 95% CI 0.45–0.94. There were no significant differences in the risk between FET and fresh groups when evaluating: pre-eclampsia (59,450 singleton pregnancies evaluated) – aOR 1.11, 95% CI 0.77–1.59; and placenta previa (69,486 singleton pregnancies evaluated) – aOR 0.70, 95% CI 0.46–1.08. The following outcomes were increased in the FET group: PIH (48,926 singleton pregnancies evaluated) – aOR 1.82, 95% CI 1.24–2.68); placenta accreta (48,158 singleton pregnancies evaluated) – aOR 3.51, 95% CI 2.04–6.05.
Limitations, reasons for caution: Although this is the largest study to date comparing the obstetric outcomes of pregnancies arising from FET and fresh cycles, it is based on observational studies and therefore subject to bias. Further studies are needed to better understand the effects of COS and cryopreservation upon mothers and offspring health.
Wider implications of the findings: It is necessary to balance the pros and cons between FET and fresh cycles. However, if the potential benefit of FET over fresh cycles regarding obstetric outcomes is confirmed in more studies, it will be feasible to change the routine of IVF/ICSI treatments.
Trial registration number: PROSPERO CRD42015029800

P-557 Is trial registration an indicator of risk of bias in fertility trials?

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Study question: Are there differences in the methodological quality and epidemiology of unregistered and registered trials of fertility treatments?
Summary answer: Registered fertility trials were considered low risk for randomisation, allocation concealment, and for non-reporting the prestated primary outcomes.
What is known already: The International Committee of Medical Journal Editors stated that from 2005 all trials should be registered. This resulted in an increase in the number of registered trials. However unregistered trials are still being published and biased, low quality publications remain problematic for systematic reviewers. It has been suggested that unregistered trials are of lower methodological quality than registered, and therefore should not be included in systematic reviews in order to increase validity. However there is no published evidence that unregistered trials negatively impact on the quality of evidence. Another issue is whether excluding unregistered trials increase the likelihood of publication bias.

Study design, size, duration: A search was performed in the Cochrane Gynaecology and Fertility Group's specialised register for all full text, English language and subfertility trials from 2010 to 2014. 706 were included in the study while 232 were excluded. We checked for registration firstly in the paper, the clinical trials registries and then by contacting the authors. Data were then extracted, the variables collected included country of primary author, fertility topic, sample size, funding and the journal where published.
Participants/materials, setting, methods: A random selection using a computer generated list of trials stratified by year (2010–2014) of 25 unregistered and 25 registered in each year was undertaken, 250 in total. Trials were assessed using the Cochrane risk of bias tool where the domains of randomisation, allocation concealment, blinding, attrition, and selective reporting were classified as low or high/unclear risk (combined) and odds ratios with 95% confidence intervals were reported. Authors were contacted for additional information where necessary.
Main results and the role of chance: The top 10 fertility journals saw an increase in registered trials from 46% to 58%. Although in the two leading journals the rate in 2014 was 90%. Of the included trials 44% were registered. For each year there were more unregistered trials published in journals than registered. Registered trials were more likely to have low risk of bias for randomisation (OR 2.47 95% CI 1.42–4.28), allocation concealment (OR 2.23 95% CI 1.30–3.83) and for non-reporting the prestated outcome from the protocol (OR 57.69 95% CI 19.94–166.87). There was no evidence of difference between registered and unregistered trials in low or high risk categories for blinding or incomplete outcome data. Unregistered trials were more likely to have a high/unclear risk of bias in selective reporting of expected outcomes (OR 0.50 95% CI 0.30–0.83). The six countries publishing the greatest number of trials were Iran (n = 113), USA (n = 76), Egypt (n = 73), Italy (n = 61), China (n = 56) and Turkey (n = 52). The proportions for registered and unregistered trials are; Iran (63%/37%), USA (58%/42%), Egypt (44%/56%), Italy (21%/79%), China (34%/66%) and Turkey (15%/85%). Funding sources were not stated in 70% of unregistered trials and 47% of registered trials.
Limitations, reasons for caution: We were unable to contact all authors for additional information. Trials were not assessed to discover if unregistered trials were more likely to have positive results. Sample sizes were low so we analysed only the 10 most frequently occurring journals in our sample and we only included English language trials.
Wider implications of the findings: Inclusion of unregistered trials in systematic reviews may affect the methodological quality of systematic reviews. However, a significant proportion of trials are still unregistered and their exclusion will reduce the number of trials that are included in a meta-analysis. Journals should insist on manuscripts that have trial registration.
Trial registration number: Not applicable.

P-558 Cumulative live birth rate following IVF and ICSI: a population-based cohort study

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Study question: What is the cumulative live birth rate following *In Vitro* Fertilisation (IVF) cycles compared with Intracytoplasmic Sperm Injection (ICSI) cycles?
Summary answer: ICSI resulted in a similar live birth rate to IVF for couples with male factor infertility but was lower for couples with non-male infertility.
What is known already: The ICSI procedure was developed for couples with male factor infertility. There has been an exponential increase in the use of ICSI regardless of the cause of infertility. Cycle-based statistics show that there is no difference in pregnancy rates between ICSI and IVF when the subfertility is not due to a male factor. However, evidence indicates that ICSI is associated with increased risk of adverse perinatal outcomes. There is little patient-based population data on the cumulative live birth rate for ICSI compared to IVF when the subfertility is not due to a male factor.
Study design, size, duration: A population-based cohort of women who had their first stimulated cycle between July 2009 and June 2013 in Victoria, Australia was evaluated retrospectively. Characteristics and treatment outcomes for women undergoing IVF cycles (n = 3730) and ICSI cycles (n = 7153) were compared. Treatment and clinical outcomes were recorded for the first autologous stimulated cycles and associated thaw cycles women had until June 2014, or until a live birth was achieved.
Participants/materials, setting, methods: Demographic and treatment related information was obtained from the Victorian Assisted Reproductive Treatment Authority, a statutory authority which records details of all ART treatments undertaken in the state of Victoria, Australia. A generalized estimating equation model was used to compare the cumulative live birth rate between the two groups. Adjustment was made for maternal age, cause of infertility, parity, number of oocytes retrieved and number of embryos transferred.
Main results and the role of chance: The 3730 women undergoing IVF cycles and 7153 women undergoing ICSI cycles had 5938 and 11153 embryo transfer cycles, which resulted in 1382 and 2493 live deliveries, respectively. The mean age of women in the IVF group was 35.1 ± 4.7 years and 34.8 ± 4.9 years for those in the ICSI group ($p < 0.05$). For couples with known cause of infertility, male factor infertility (male factors only or combined male/female factors) was reported for 35.9% in the IVF group and 62.7% in the ICSI group. Non-male factor infertility (female factor infertility only or unexplained infertility) was reported for 64.1% in the IVF group and 37.3% in the ICSI group ($p < 0.01$). Fertilisation rate per oocyte treated was higher in the ICSI group (70.1%) than in the IVF group (60.3%) ($p < 0.01$). The cumulative live birth rate within six cycles was 36.9% for IVF the group and 34.8% for ICSI group. Among couples with male factor infertility, ICSI resulted in a similar cumulative live birth rate (Adjusted odds ratio (AOR): 0.88, 95% confidence intervals (CI): 0.73–1.06) compared with IVF. For couples with non-male factor infertility, ICSI resulted in a significantly lower cumulative live birth rate (AOR: 0.76, 95% CI: 0.64–0.90) compared with IVF.
Limitations, reasons for caution: The reported causes of infertility were based on the treating clinician's classification and criteria may have varied between clinicians. Details of infertility diagnosis and indication for ICSI were not available. Residual confounders might have impacted the findings of this study since not all potential confounders were collected in the dataset.
Wider implications of the findings: This population-based study found a higher cumulative live birth rate following IVF compared with ICSI among couples with non-male factor infertility. It suggested that ICSI offers no advantage over IVF in terms of live birth rate for couples with non-male factor infertility.
Trial registration number: NA.

P-559 Development of an external quality assessment (EQA) scheme for embryo morphology using the UK standard embryo grading scheme

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Study question: EQA for embryology morphology launched in 2011 as part of the UK National External Quality Assessment Service (UK NEQAS) for Reproductive Science.
Summary answer: Currently 60 UK laboratories (>80% of licensed assisted conception units) and 30 international laboratories participate in the EQA scheme for embryo morphology.
What is known already: The primary aim of UK NEQAS is educational, with 390 pathology schemes operating (not for profit) from 26 centres. EQA scheme participants receive independent, objective and impartial reports on their performance, enabling them to identify weakness and take appropriate action. UK NEQAS Reproductive Science includes EQA for Andrology launched in 1994 (currently with 200 UK and 90 international participants). EQA for embryology followed as an on-line scheme in 2011 with all participants using the UK embryo grading scheme (Cutting et al., 2008).
Study design, size, duration: Data was taken from EQA scheme participants for inter-laboratory comparison of embryo morphology assessment between April 2011–December 2015. In addition, a survey of UK embryologists in January 2016 asked for feedback on: (i) participation in the EQA scheme; (ii) routine use of the UK embryo grading scheme; (iii) the need for review/update

of the UK embryo grading scheme and (iv) interest in EQA for time-lapse imaging annotation.

Participants/materials, setting, methods: Laboratories assessed embryo images four times per year on line (Gamete Expert website). A single set of results (from one "assigned" embryologist per lab) was submitted for each distribution; including four each of day 2, 3 and 5 stage embryos (1 min "rolling" embryo videos); plus 2 time lapse videos from 1 cell to blastocyst stage. Target values were derived from an all laboratory consensus and an EQA performance report was produced for each laboratory.

Main results and the role of chance: Between April 2011–December 2015, a total of 264 embryo images (152 day 2/3 videos and 112 day 5 videos) were distributed over 19 occasions. Least agreement between laboratories was found for blastocyst inner cell mass grading with a consensus (used to derive target values) reached for only 70% (78/112) of embryos; 80% of blastocysts reached consensus for expansion and 96% for trophectoderm grading. Consensus was reached for 99% of day 2/3 embryos assessed for degree of fragmentation, 95% for cell number and 90% for evenness/cell size.

78 UK embryologists responded to the embryo grading survey; 87% (68/78) participated in the EQA scheme; 44% (30/68) of those in the scheme also participated with individual logins; 59% of respondents routinely used the UK grading scheme for cleavage stage embryos and 65% for blastocysts; the remainder mainly used "in-house" grading schemes. The majority of respondents (88%) would like to see a review of the UK standard grading scheme. Although only 41% (32/78) of respondents routinely used time-lapse imaging, 82% (56/68) of those in the EQA scheme were interested in EQA for time-lapse annotation and 6/10 labs not already participating would join the scheme if this was offered.

Limitations, reasons for caution: Alternative methods to derive "target values" for comparison of inter- laboratory performance could be considered. Only around 20% of the ACE membership responded to the embryo grading survey and the results may not be representative of all UK embryologists.

Wider implications of the findings: Increasing the number of EQA participants would provide a larger data set for inter-laboratory comparisons, allowing for further development/improvement of the scheme and ultimately grading standardization. The interest shown in EQA for time lapse annotation merits its inclusion in the scheme and a pilot study is planned.

Trial registration number: Not applicable.

P-560 Perinatal death after fertility treatment; a 7-year national cohort study in New Zealand on contributory factors and potential avoidability

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Study question: What are the causes of and contributory factors to perinatal death after fertility treatment in New Zealand, and how many deaths were potentially avoidable?

Summary answer: Main causes of perinatal death were congenital abnormalities and specific perinatal conditions. Contributory factors were found in 10%; 4% of cases were considered potentially avoidable.

What is known already: Available international data on perinatal mortality from large population based fertility treatment cohorts, provides information on common risk factors such as obesity, smoking and fetal growth restriction. Very few studies however provide data on the underlying causes of death or potentially avoidable deaths.

As fertility pregnancies are "planned pregnancies," the causes of death may differ, and it is important to know if any of the deaths were potentially avoidable and if there were modifiable causes such as specific treatment regimens.

Study design, size, duration: All perinatal deaths in New Zealand (i.e., fetal death from 20 weeks gestation and neonatal death up to 27 days) are centrally collected by the Perinatal and Maternal Mortality Review Committee (PMMRC). We undertook a retrospective, 7-year cohort study (Jan 2007–December 2014) to analyse all perinatal deaths in New Zealand of babies conceived after fertility treatment including ovulation induction (OvI), intra uterine insemination (IUI) and *in vitro* fertilisation (IVF) and intra-cytoplasmatic sperm injection (ICSI).

Participants/materials, setting, methods: A multidisciplinary PMMRC local panel reviewed each perinatal death and identified the cause of death, contributory factors and potential avoidability. We analysed characteristics for all mothers, deceased babies and singleton and multiple gestations conceived after OvI, IUI or IVF/ICSI. Substandard factors directly related to the fertility treatment, or related to the fact that this was a "planned" pregnancy, were collected.

Main results and the role of chance: In total, 261 perinatal deaths from 234 pregnancies in 232 mothers were included. There were 168 singleton deaths and 93 deaths from 66 multiple gestations (55 twins, 8 triplets and 3 quadruplets). Most pregnancies were conceived by IVF/ICSI (71%, including 6% after oocyte donation) followed by clomiphene (22%) and IUI (7%). Most multiples derived from IVF/ICSI (n = 50, 75%) followed by 12% after clomiphene, which also accounted for most high-order gestations. Maternal risk factors such as BMI >32, alcohol drug or tobacco abuse, were low. 53% were born <24 weeks gestation, followed by 14% <27 weeks; 33% were small, 6% large and 45% appropriate for gestational age. 22% of deaths were terminations of pregnancy, 54% stillbirths and 25% neonatal deaths. Main causes of death were congenital abnormalities (26%), specific perinatal conditions (24%, e.g., cervical incompetence, twin to twin transfusion or complication of intra-uterine procedures), spontaneous preterm labour (15%), antepartum haemorrhage (10%).

In-depth local case review reported contributory factors in 24 pregnancies (10%, 16 singleton, 8 multiples), mainly relating to "personnel" and "organisation & management of care." 15 deaths (4%) were considered potentially avoidable. For 7 cases, avoidability was related to fertility treatment and concerned high-order multiples.

Limitations, reasons for caution: Retrospective analysis of perinatal mortality data does not provide insight into "near-miss" cases, substantial morbidity or infant death beyond 27 days. Moreover, the denominator for some substandard factor analyses is unknown, as non-fatal cases are not included in a New Zealand registry.

Wider implications of the findings: To our knowledge, this is the first nationwide confidential review into perinatal deaths after fertility treatment. Recognition of contributory factors is important to increase understanding of perinatal death after fertility treatment. It could facilitate feedback to professionals and education of the public on, e.g., the risks of multiple pregnancies.

Trial registration number: Not applicable

P-561 Assisted reproduction causes reduced fetal growth associated with downregulation of paternally expressed imprinted genes that enhance fetal growth

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Study question: Whether manipulation of embryo affects genomic imprinting and developmentally relevant genes in mouse placenta and resulted in fetal reduced growth at E10.5.

Summary answer: ART placentae exhibited perturbed genomic imprinting and the expression of developmentally relevant genes and functional genes for nutrition transport was also affected.

What is known already: ART affects placental nutrient transport and results in lower fetal weight and placenta overgrowth at E18.5 in mice. ART placentae also exhibited histomorphological alterations with defects in placental layer segregation and abnormal migration of glycogen cells at E18.5 in mice, and perturbed genomic imprinting may contribute to these problems.

Study design, size, duration: Mouse blastocyts were produced by (1) embryo culture *in vitro* after *in vivo* fertilization (IVC) or (2) *in vitro* fertilization (IVF), and (3) *in vivo* fertilization and development and then transferred to the uteri of pseudopregnant females. The fetal crown-rump length, placental weight, methylation level of *H19*, *KvDMR1* and *SNRPN*, expression level of multiple imprinted genes and genes that important for placental development were compared between the *in vivo*, IVC and IVF groups at E10.5.

Participants/materials, setting, methods: Virgin 6- to 8-week-old CD1 female mice, adult CD1 males were used. DNA bisulfite treated using the EpiTect Bisulfite Kit (Qiagen). DNA methylation was analyzed by MassARRAY platform (Sequenom). Real-time PCR was performed using the CFX96 real-time PCR instrument (Bio-Rad). Data were subjected to analysis of variance test for assessing any significant difference. The least significant difference post hoc

test was used to examine any significant difference between groups. *P*-values <0.05 were considered significant.

Main results and the role of chance: ART procedures could reduce fetal and placental growth at embryonic day 10.5. Moreover, ART lead to decreased methylation levels at *H19*, *KvDMR1*, and *SNRPN* imprinting control regions in placentae ($P > 0.05$), instead of fetuses. Furthermore, in the placenta, ART resulted in the downregulation of a majority of parentally expressed imprinted genes (*Dlk1*, *Igf2*, *Kcnq1ot1*, *Ndn*, *Peg3*, *Plagl1*, *Sgce*, *Peg10*, *Peg11*, *Zdbf2* and *Sfmbt2*), whereas it resulted in the upregulation of a majority of maternally expressed genes (*Cd81*, *Cdkn1c*, *Dcn*, *Gnas*, *H19*, *Mash2*, *Gatm*, and *Phlda2*). Additionally, the expression of genes (*Gcm1*, *Syna*, *Synb, Prl8a8, Tpbp*a, *Pcdh12, Hand1, Gys2*, *Gbe1, Hif1*a, *Hsd11b2, Mapk14* and *Stat4*) that regulate placental development was also affected by ART. Last but not the least, ART resulted in the downregulation of a majority of placental nutrient transporters (*Slc38a1, Slc38a4, Atp2a3, Atp1a1, Slc1a3, Slc6a6, Slc19a2, Slc26a7, Slc22a3* and *Slc22a18*).

Limitations, reasons for caution: This study was carried out using a mouse model. Whether ART affects the development and function of human placenta, and influences the growth of fetus in early pregnancy need further study.

Wider implications of the findings: The results from this study suggest that ART disrupts epigenetic reprogramming events and perturbs developmental gene expression in the mouse placenta that may affect the placental development and function, which may affect fetal growth and reprogramming.

Trial registration number: no

P-562 Am I ready for ICSI? Individualized training for laboratory technicians and assessment of learning curves

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Study question: Can an individualized ICSI training, tailored in realtime to the trainee performance, allow technicians with no experience in embryo manipulation to perform ICSI effectively?

Summary answer: Learning ICSI requires a variable number of attempts; however, following training with the proposed system, technicians can perform real ICSI as proficiently as senior embryologists.

What is known already: ICSI is widely used and central to the efficiency of ART cycles. ICSI is also operator-dependent, and no standardized training and evaluation of competence achievement for technicians is currently available. Although most laboratories establish internal training schemes to learn ICSI, they are not tailored to the trainee and do not allow individualized assessment of proficiency. Moreover, the vast majority of training schemes are not validated against clinical results. The present study aims to establish an individualized ICSI training for ART technicians without previous experience in micromanipulation, and to evaluate their training performance.

Study design, size, duration: Prospective study including 5 laboratory technicians. ICSI training consisted of 1 theoretical phase common to all technicians, and 1 individualized. The individualized part consisted in performing ICSI with latex microsphere (LM) on discarded oocytes. Injected oocytes outcomes were recorded and analyzed in real time in order of injection. Learning curve-cumulative summation (LC-CUSUM) curves were used to monitor in real-time when ICSI competence was achieved. Post-training performance against senior embryologists was assessed by Student' *t*-test.

Participants/materials, setting, methods: The LM inside the oocyte 24 h post injection was considered a successful ICSI. A lysed oocyte, or the LM placed outside of the plasmalemma, were considered failures. In a LC-CUSUM graph, each success moves the learning curve towards the decision limit, while each failure moves it back towards the *x*-axis. The trainee is not proficient until the learning curve crosses the decision limit. A trainee was considered trained when his failure rate remained within 5%.

Main results and the role of chance: Four technicians trainees became proficient at ICSI after a variable number of LM injections, ranging from 35 to 80. This allowed for the lab to move trainees to clinical practice after different times spent training, while all achieved the same level of proficiency. One trainee did not achieve competence after 80 LM injections and was retrained under the supervision of a senior embryologist. All technicians that achieved proficiency with LC-CUSUM, whatever the number of ICSI with LM necessary to do so, went on to perform ICSI with human gametes in a clinical setting as proficiently as senior embryologists, measured by their lysis, fertilization and rate of number of viable embryos available for ET in the first 50 real cases post-training. LC-CUSUM based, personalized training was therefore validated against clinical cases and senior embryologists. All trainees were women and all has some experience in simple ART techniques such as washing semen samples, but never handled oocytes or used a manipulator.

Limitations, reasons for caution: In this study, ICSI competence is acquired after a specific training through a structured theoretical and practical course followed by personalized learning curve. The observed results cannot be generalized to other training programs. The proposed system can be applied widely given the absence of animal gametes and human fertilization.

Wider implications of the findings: A variable number of ICSI are needed to achieve proficiency; conventional ICSI trainings might undertrain technician, undermining cycle efficiency for the patients. A tailored and continuous individualized assessment of training optimizes both training time and resources, and identifies technicians that cannot learn effectively.

Trial registration number: NA

P-563 Separated double embryo transfer represents a novel method in assisted reproductive technology treatment to prevent multiple pregnancies

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Study question: Does a novel embryo transfer method in which two embryos are separately transferred (s-DET) prevent multiple pregnancies than does conventional double-embryo transfer (c-DET)?

Summary answer: The incidence of multiple pregnancy rates in the s-DET group was significantly lower than that in the c-DET group.

What is known already: Previous studies have reported that transferred embryos remain localized to the areas in which they were initially transferred. Furthermore, approximately 80% of the embryos implant into the direct area in which the catheter tip was placed, as determined by the ultrasonic observation of air bubbles from the catheter following embryo transfer. Moreover, the implantation rate of DET with two morphologically good embryos (MGEs) has been shown to be significantly lower than that of SET with only one MGE. These data suggest that embryos may interfere with each other when several embryos are trying to implant within the same restricted space.

Study design, size, duration: This was a prospective cohort study. A total of 231 patients with a history of two or more failed implantations after fresh or thawed ET cycles were consecutively enrolled between May and December 2015. This study was approved by our Institutional Review Board, and all patients provided informed consent upon enrollment.

Participants/materials, setting, methods: A total of 137 patients underwent s-DET (s-DET group, in which two embryos were transferred separately), whereas 94 patients underwent c-DET (c-DET group, in which two embryos were transferred together. In both groups, either fresh or frozen-thawed embryos were used. A range of reproductive outcomes were compared between the groups, including gestational sac development, clinical pregnancy, implantation, multiple pregnancy, and miscarriage rates.

Main results and the role of chance: The average age of the s-DET group was 39.2 ± 3.2 (mean ± SD) years, which was comparable to that of the c-DET group (39.1 ± 3.9 years). The history of pregnancy and delivery in the s-DET group was similar to that in the c-DET group. The number of previous ET attempts in the s-DET group was 3.7 ± 3.4 (mean ± SD), which was comparable to that in the c-DET group (2.6 ± 2.5). Clinical pregnancy and implantation rates in the s-DET group were 25.3 and 15.1%, respectively, which were comparable to those in the c-DET group (27.6 and 17.5%, respectively). Multiple pregnancy rate was significantly lower (2.9%) in the s-DET group than in the c-DET group (25.0%; $P < 0.01$).

	s-DET	c-DET	*P* value
Number of cycles	137	94	
Age, years	39.2 ± 3.2	39.1 ± 3.9	
Number of previous ET attempts	3.7 ± 3.4	2.6 ± 2.5	
Number of transferred embryos, *n*	274	188	
Number of clinical pregnancies, *n* (%)	35 (25.3)	26 (27.6)	NS

GS, *n*	36	33	NS
Implantation rate, %	15.1	17.5	NS
Number of multiple pregnancies, *n* (%)	1 (2.9)	7 (25.0)	0.009
Number of miscarriages, *n* (%)	5 (14.2)	6 (23.1)	NS

Limitations, reasons for caution: The small sample size of the s-DET group represented one potential limitation. Furthermore, implantation is believed to be dependent upon an intimate relationship between the embryo and uterine tissues mediated by locally secreted factors. Determining the mechanism underlying these findings requires further research.
Wider implications of the findings: An embryo that implants first may attempt to maintain an appropriate distance from its neighbor to prevent overlap, thus interfering with the other embryo's ability to implant. This could explain the significantly higher multiple pregnancy rate in the c-DET group than in the s-DET group.
Trial registration number: None

P-564 Is transfer on blastocyst stage related to a higher risk of pregnancy complications?

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Study question: To determine whether the obstetrical complications are more frequent after blastocysts stage transfer (day 5 or 6) versus embryo transfer on day 2 or 3.
Summary answer: The main factor for the appearance of obstetrical complications (i.e., gestational diabetes, pregnancy induced hypertension or premature rupture of membranes) is transfer on blastocyst stage.
What is known already: The blastocyst stage transfer (day 5 or 6) is a relatively new technique with theoretical advantages over day 2 or 3 transfer: it is more physiological as it is more similar to what happens in natural conditions, the implantation rate is higher as only the viable embryos reach the blastocyst stage, it allows a good selection of the best embryo to transfer and there is a better embryo-endometrium synchrony.

Despite the known advantages of day 5 or 6 embryo transfer, the effect on the pregnancy and the new born development remains unclear.
Study design, size, duration: This is a cross sectional study where pregnancies obtained after *in vitro* fertilization (IVF) or donor oocytes cycles were included. The study period of time was 2013 and 2014.

A total of 374 pregnancies were included. 217 of them were obtained after donor oocyte treatment and 157 after IVF. In 123 cases, the embryos were transferred on blastocyst stage and in 251 cases on day 2 or 3.
Participants/materials, setting, methods: χ^2 test was performed to compare the obstetrical complications in blastocyst stage transfer and non- blastocyst stage one. The obstetrical complications studied were: gestational diabetes, pregnancy induced hypertension and premature rupture of membranes.

We used a multivariate logistic regression model to avoid confounding factors in the obstetrical outcomes such as age, type of treatment, transfer on blastocyst stage and twin pregnancy.
Main results and the role of chance: In our study group, we found statistically significant differences between transfer on blastocyst stage and some of these obstetrical complications: gestational diabetes (11.5 versus 4.9 p 0.008), pregnancy induced hypertension (16.7 versus 7.4 $p = 0.002$) and premature rupture of membranes (16.7 versus 7.1 $p = 0.001$)

When we analyzed confounding factors such as age (<38 years old versus >38 years old), type of treatment (IVF versus donor oocytes treatment), single pregnancy versus twin pregnancy and transfer on blastocyst stage or not, the only factor found with a statistically significant relation with the appearance of the studied pregnancy complications was the transfer on blastocyst stage with an Odds Ratio (OR) 2.34 ($p < 0.001$).

Regardless of age, type of treatment or the presence of twin pregnancy, transfer on blastocyst stage increases the risk for obstetrical complications.
Limitations, reasons for caution: Due to the statistical design, more studies are needed to advice suitably our patients about the transfer on blastocyst stage implications
Wider implications of the findings: In the study patients, we found a higher risk of obstetric complications such as gestational diabetes, pregnancy induced hypertension and premature rupture of membranes in those patients with day 5 or 6 embryo transfer.

More studies are needed to confirm our findings.
Trial registration number:

P-565 Prevalence of ovarian hyperstimulation syndrome (OHSS) and hypercoagulability in patients triggered by GnRH agonist for excessive follicular response: a systematic follow-up

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Study question: In patients with excessive follicular response after ovarian stimulation with antagonist protocol, does GnRH agonist triggering and "freeze-all" strategy prevent the occurrence of OHSS?
Summary answer: Triggering by GnRH agonist and "freeze-all" strategy does not fully prevent the occurrence of OHSS, but limits its duration.
What is known already: In the literature, the risk of moderate to severe OHSS is 3 to 6% and reaches 31% in high risk populations 9 days after oocyte triggering with hCG. Many studies report no or a markedly decreased risk of OHSS after triggering ovulation with a GnRH agonist. However, criteria to define OHSS are rarely explained and OHSS itself is not thoroughly asserted. It is well known that OHSS is associated with hypercoagulability. However, no study after triggering with a GnRH agonist assessed haemostasis in these high-risk patients with high circulating estradiol levels.
Study design, size, duration: In a French academic reproductive medicine centre, a systematic prospective observational follow-up of all patients triggered by GnRH agonist for excessive follicular response was conducted. 52 patients were included from January 2014 to July 2015.
Participants/materials, setting, methods: All patients undergoing antagonist protocol and at high risk of OHSS (estradiol level ≥3000 pg/mL and/or more than 20 follicles ≥11 mm on the day of triggering) were triggered by GnRH agonist. No luteal phase support and a "freeze-all" strategy were performed. On the day of oocyte retrieval (T0), at 48 h (T1) and at day 7 (T2), OHSS and hypercoagulability were systematically assessed. Haemostasis data were compared to the initial status of each patient.
Main results and the role of chance: The prevalence of moderate to severe OHSS was respectively 26.9% (T0) (14/52), 15.38% (T1) (8/52), and 7.6% (T2) (4/52). As a whole, 18 patients had moderate to severe OHSS during follow-up (34.6%). Only 1 patient had severe OHSS requiring hospitalisation. No difference in clinical or ovarian stimulation parameters was observed between the patients who experienced OHSS and the others. Hypercoagulability was evidenced at each stage of the follow-up but no thromboembolic event happened. A rapid decrease of estradiol and progesterone levels was observed, reaching basal levels at T2. Finally, pregnancy rate by oocyte retrieval was 26.9%.
Limitations, reasons for caution: OHSS classification is not precise. Subjective criteria are included and the number of criteria necessary to move from one stage to another is not well described, which could lead to misevaluation of OHSS and difficulties of comparison to the literature.
Wider implications of the findings: OHSS prevalence is much higher than expected. The occurrence of one case of severe OHSS is consistent with the fact that GnRH agonist triggering does not completely prevent from OHSS. Hypercoagulability appears after GnRH agonist triggering for excessive follicular response and a thrombosis prophylaxis must be discussed.
Trial registration number: CPP Nord-Ouest 4: CPP16/03

P-566 Perinatal outcome of children born after vitrification of blastocysts (9480 cycles with 3143 babies in 16 years experiences)

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Study question: We analyzed perinatal information in infants born after vitrified blastocysts transfer including congenital problems after 13 weeks of gestation, and the rate of monozygotic twinning (MZT).
Summary answer: The perinatal results of 2849 births with 3143 babies, rate of congenital defect and perinatal complication (1.9%) confirm the safety of vitrified BL program.
What is known already: Since vitrification approach has been widely recognized as an established method for human oocyte and embryo cryopreservation, better clinical outcome than the one of classical slow cooling method has been often reported. Recent attention has been paid to the safety of this vitrification approach.
Study design, size, duration: Between 2000 and 2014, a total of 14370 vitrified BLs from 9480 cycles were warmed and 13748 survived (95.7%). In 9363 vitrified BLs transfers, 4447 resulted in pregnancy (47.5%). A total of 12665 vitrified BLs were transferred and 4815 were implanted (38.0%). In 2849 births 3143 babies (1655 boys & 1488 girls) were born.
Participants/materials, setting, methods: Vitrification involved the exposure DMSO and EG during the cooling step, and 0.25/0.5 M sucrose solution for warming. The blastocoelic cavity was collapsed before vitrification, and after warming, zona hatching was carried out with laser pulse. MZT was defined the number of FHB was more than that of embryos transferred.
Main results and the role of chance: Fifty two clinical pregnancies were confirmed as MZT (1.8%). Forty six cases had either congenital birth defects or perinatal complication (1.5%), including eight chromosomal abnormalities (two 18 trisomy, six 21 trisomy), nine multiple anomalies, one stillbirth due to hydropus, seven stillbirths of unknown causes during delivery (25, 29, 30, 32, 37, 38, 39 weeks of gestations), two anencephaly, one spina bifida, eleven congenital heart or major vessel malformations and three minor anomalies in hands and/or feet, one congenital esophageal obstruction, one biliary duct obstruction, one Cornelia de Lange syndrome (CdLS), and one Treacher Collins syndrome. No statistical difference was seen in the mean gestational days of between fresh BT (272.5 days) and vitrified BT (273.8 days). However, significant difference was observed in the mean birth weight of full term birth with fresh BT (2998 ± 371.5) and vitrified BT (3097 ± 406.2).
Limitations, reasons for caution: Results of vitrified BLs program were compared with the one of fresh BT program in the same period. However, background of both groups may not be completely same due to private clinical set-up the program.
Wider implications of the findings: Also, the high implantation rate encourages us to establish single BL transfer. Further investigation of developmental competence of childrens born after vitrified BL will be necessary.
Trial registration number: None

P-567 Behavioural, cognitive, and motor performance of children born after intracytoplasmic sperm injection with testicular sperm at the age of five

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Study question: What is the frequency of behavioural, cognitive, motor or physical problems of children born after intracytoplasmic sperm injection with testicular sperm (TESE-ICSI) at the age of five?
Summary answer: Five out of 86 children (5.8%) had developmental problems based on two or more of behavioural, cognitive and motor assessments, or based on previous diagnosis.
What is known already: A number of studies described pregnancy and neonatal outcome, focussing mainly on congenital malformations in children born after TESE–ICSI up until two years of age. No studies have been performed on behavioural, cognitive and motor performance of children born after TESE–ICSI above the age of two.
Study design, size, duration: We performed a prospective longitudinal cohort study including 378 live born children after TESE-ICSI. Data were collected between March 1st, 2008 and January 1st, 2016 in the fertility centres of the Radboud university medical center in Nijmegen and the Academic Medical Center in Amsterdam, The Netherlands. In this period, 108 children reached the age of five. In total 86 children were part of the "5-years-old cohort" while 22 (20.4%) were lost to follow up.
Participants/materials, setting, methods: Questionnaires were sent at birth, and at one and four years of age. Five-year-old children were invited for assessment. Behavioural performance was assessed using the Child Behaviour Checklist for parents and teachers. Cognitive performance was assessed using the Wechsler Preschool and Primary Scale of Intelligence test, third version. Motor performance was assessed using the Movement Assessment Battery for Children, second version. We performed also a physical examination.
Main results and the role of chance: Of the 108 five-year-old children, 79 children were completely assessed and seven were partially assessed at the age of five. Behaviour was in the normal range: the child behaviour checklist of the mothers, fathers and teachers reported behaviour in the normal range in 73/80 (91.3%), 77/80 (96.3%) and 65/74 (87.8%), respectively. Intelligence level was also in the normal range: the total IQ was mean 111.1 with a standard deviation of 11.7. In 62/80 (77.5%) we found a normal motor performance. The mean total test score for motor performance was 9.5 with a standard deviation of 3.1. Five children (5/86; 5.8%) of the five-years-old cohort had developmental problems. Two of them were previously diagnosed with a form of autism (Pervasive Developmental Disorder-Not Otherwise Specified). Three children had developmental problems based on two or more of our behavioural, cognitive and motor assessments.
Limitations, reasons for caution: In this study we found that five children had developmental problems. This study is small to draw conclusions about the relation between the mode of conception and the developmental problems.
Wider implications of the findings: Our findings seem can inform couples who consider TESE-ICSI treatment about the development of children born after this procedure.
Trial registration number: None

P-568 "Economic evaluation of single versus double embryo transfer in IVF/ICSI: a systematic review"

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Study question: Is elective single-embryo transfer (eSET) more cost effective than double embryo transfer (DET)?
Summary answer: Cost-effectiveness studies of different embryo transfer strategies present diverse levels of quality. Taking into account only high-quality studies, an eSET strategy is considered more cost-effective.
What is known already: Multiple pregnancies lead to complications and provoke high costs. eSET is the most effective strategy to reduce the multiple pregnancy rate in IVF/ICSI. In terms of cost-effectiveness, eSET could counteract the decreased live birth rate by reducing the rate of multiple pregnancies. Current opinion is that eSET success rates depend on two factors: suitable patient selection and the efficiency of the embryo cryopreservation technique.
Study design, size, duration: The protocol design followed the Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) guidelines for reporting systematic reviews and meta-analyses. The search was performed during January 2015 by two independent reviewers, applying as inclusion criterion that studies should describe a full economic evaluation. Drummond's checklist of 10 items was utilised to determine whether the method in each study was methodologically adequate for the objectives proposed and whether the results were valid
Participants/materials, setting, methods: The search for economic evaluations to be included in this eSET vs. DET review was conducted using the following databases: Medline, EMBASE, NHS Centre for Reviews and

Dissemination Database of Abstracts of Review of Effectiveness (NHS CRD DARE), NHS Centre for Reviews and Dissemination Health Technology Assessment (NHS CRD HTA), NHS Centre for Reviews and Dissemination Economic Evaluation Database (NHS CRD EED), the Cochrane Library and the US clinical trials registry.
Main results and the role of chance: Out of 137 non-duplicate studies, 126 were excluded. Thus, 11 studies met the eligibility criteria and had extractable data, and were included in the systematic review. Among these 11 studies, 18 different scenarios were analysed. The cost-effectiveness results obtained are expressed as a relative percentage. Overall, the studies reported relative reductions of 10% in the live birth rate and 15% in costs when eSET was performed. The reduction in effectiveness was not modified by differences in the quality of the studies, the perspective analysis adopted or the inclusion of cryotransfers in the studies. Neither was the cost reduction related to the perspective analysis adopted or to the inclusion of cryotransfers in the study design. However, a greater reduction was reported in "top quality" studies than in other categories (−31.8% vs. −3.8% $p < 0.05$). This direct relationship between the quality of the study and the cost reduction achieved with eSET was also observed when only studies that took cryotransfers into account were analysed (−29.0% vs. −2.7% $p < 0.05$).
Limitations, reasons for caution: For a full economic evaluation to be considered of high methodological quality, it should include an analysis from a social perspective, apply discount rates, base its findings on the incremental cost-effectiveness ratio, incorporate a probabilistic sensitivity analysis and address a long time horizon.
Wider implications of the findings: A repeated eSET strategy can minimise the risk of multiple pregnancy in couples undergoing IVF/ICSI and reduce costs, without substantially reducing the likelihood of achieving a live birth. Most studies of this question included young women with a good prognosis.
Trial registration number: No trial registration number.

P-569 Therapeutic effect of acupuncture on the outcomes of *in vitro* fertilization: evaluated by an updated systematic review and meta-analysis

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Study question: This study aim to evaluate the effect of acupuncture on *in vitro* fertilization (IVF) outcomes.
Summary answer: Acupuncture improves CPR among women undergoing IVF based on all the studies.
What is known already: Although *In vitro* fertilization (IVF) increases success rate, its repeated cycles does not only cause enormous economic pressure on the patients and their families, but also invasive and time consuming. Therefore, new therapies which can improve reproductive outcomes are highly desirable. Acupuncture as one of the oldest, most commonly used medical procedure in the world, while the effect of acupuncture on IVF-ET outcomes still remain to be explored.
Study design, size, duration: This study is systematic review and meta-analysis. Patients are women undergoing IVF in randomized controlled trials (RCTs) who were evaluated for the effects of acupuncture on IVF outcomes.
Participants/materials, setting, methods: And the treatment groups were used by traditional, electrical, laser, auricular and other acupuncture techniques. The control groups were consisted of no, sham, and placebo acupuncture. The major outcomes were biochemical pregnancy rate (BPR), clinical pregnancy rate (CPR), live birth rate (LBR), ongoing pregnancy rate (OPR). Heterogeneity of the therapeutic effect was evaluated with a forest plot analysis. Publication bias was assessed by a funnel plot analysis.
Main results and the role of chance: Thirty trials (a total of 6,344 participants) were included in this review. There were no significant publication biases for most of the comparisons among these studies. The pooled CPR (30 studies) from all of the acupuncture groups was significantly greater than that from all of the control groups. The pooled CPR from all acupuncture groups was significantly higher than the controls. Subsequently, the pooled CPR, LBR, BPR and OPR results were significantly higher in the acupuncture group than in the controls when acupunctures are conducted during controlled ovarian hyperstimulation.
Limitations, reasons for caution: The effect of acupuncture on IVF-ET is still remain to be seen based on more trails.
Wider implications of the findings: However, optimized positive effects could be expected using acupuncture in IVF during controlled ovarian hyperstimulation.
Trial registration number: No.

P-570 Do internal audits increase pregnancy rates?

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Study question: Does a time consuming internal audit in real ART Practice improve pregnancy rates?
Summary answer: Yes, internal audit holds a mirror up to us for us to clearly identify the problem areas and rectify them.
What is known already: Several published papers are available on analysis of factors predicting live birth rate, logistic regression analysis of multiple variables that affect ART outcome and identification of individual predictors of pregnancy. All these studies are over a period of several years and with more than 1000 treatment cycles. There is also software available for the analysis of cycles of an ART Practice. But, there appears to be no published study if such an analysis internally plays a role in the improvement of an ART Practice. Our study question is aimed at a small ART Practice with 250 cycles per year.
Study design, size, duration: The study was a prospective cohort study of 327 cycles in 2014 compared to 365 cycles in 2015. The outcome of an ART cycle depends on a multitude of determinable and indeterminable variables. We evaluated the maximum possible determinable variables. The entire clinical profile (15 variables), ovarian stimulation details (18 variables) combined with the laboratory data (27 variables) of the ART cycle of each patient was entered into the statistical data analysis software and analyzed.
Participants/materials, setting, methods: All patients undergoing an ART Cycle at the Universitaeres Kinderwunshzentrum in Kiel, Germany in 2014 and 2015 were included. The data of the patients was entered into the SPSS Statistical software sheet and analyzed. The 1st analysis was made in January 2015 and the second analysis in January 2016. After the 1st analysis, the effect of the changes in the clinics and in the lab and was reanalyzed in the 2nd analysis.
Main results and the role of chance: 60 variables (e.g., Stimulation protocols, number of ultrasounds/blood tests done, time of ICSI/embryo transfer, lab/clinical personal performing a procedure, etc) were evaluated against different end points (e.g., number of oocytes, percentage of mature oocytes, fertilization rate, grade A/B/C embryo rate, blastocyst conversion rate and biochemical/clinical pregnancy rate). Using SPSS, a multiple logistic regression analysis was conducted with regard to each end point taking into account the confounding factors from a clinical and laboratory point of view of each variable. In both the clinical and laboratory analysis there were many variables of insignificance and a few with significance after the 1st analysis. After a significant result, a discussion regarding the possibilities of role of alteration of our protocol followed. Few examples after the 2nd analysis are: A significant improvement in blastocyst conversion rate was seen when earlier ICSI was performed followed by single drop culture of embryos. Aliquoting of media in the lab significantly increased the rate of grade A embryos. An increase in biochemical pregnancy rate was seen after change in the embryo transfer catheter but it was not significant. There was a 10% increase in pregnancies from Blastocyst transfer compared to the previous year.
Limitations, reasons for caution: The sample size was small for any definitive conclusions. A combination of meticulous data collection, statistical prudence and "outside the box" analysis was necessary. The improvements seen after the modifications as a result of the audit could have been either due to only one change or a combination of changes.
Wider implications of the findings: End points other than pregnancy rates need to be analyzed. A regular audit of the procedures and protocols in an ART Practice is the way of the future. It is through audit that a practice can continue to do what is beneficial and change what it finds to be damaging.
Trial registration number: For this analysis, a registration was not required.

P-571 Blastocyst biopsy in PGD/PGS cycles does not increase the neonatal risk when compared to intracytoplasmic sperm injection cycles

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Study question: To investigate whether blastocyst biopsy in preimplantation genetic diagnosis (PGD)/screening (PGS) increase the risk of adverse neonatal outcomes.
Summary answer: Blastocyst biopsy did not add extra risk to neonatal outcomes.
What is known already: Blastocyst biopsy is becoming more popular in PGD/PGS because of potential better pregnancy outcome and the availability to obtain more biopsy material for detecting entire chromosome complement. However, the safety of the offspring after this strategy is still to be determined.
Study design, size, duration: This is a retrospective cohort study including all children born after blastocyst biopsy combined with frozen embryo transfer (487 singletons and 91 twins) and frozen blastocyst transfer after intracytoplasmic sperm injection (ICSI, 501 singletons and 153 twins) from January 2011 to March 2015 in Reproductive and Genetic Hospital of CITIC-Xiangya.
Participants/materials, setting, methods: All patients who received blastocyst biopsy with frozen embryo transfer (biopsy group) or frozen blastocyst transfer after ICSI (non-biopsy group) and delivery at least a live baby were enrolled in this study. Patients using donated oocytes or sperm or multifetal reduction during pregnancy or testicular sperm aspiration were excluded. In addition, all triplets were not included in the statistical analysis because of the small sample size. All embryos were applied with ICSI in PGD/PGS cycles.
Main results and the role of chance: No differences were observed in both the absolute gestational age (GA)/birthweight (BW) and the GA/BW adjusted for maternal age, BMI, etiology, arrested intrauterine pregnancy, source of sperm, fetal sex of the newborns between biopsy and non-biopsy groups. The sex ratio, rate of birth defects, preterm birth (PB), very preterm birth (VPB) as well as the rate of low birthweight (LBW), very low birth weight (VLBW) and macrosomia were all comparable between the two groups no matter in singletons or twins. Meanwhile, the neonatal outcomes including GA, BW, rate of PB, VPB, LBW, VLBW did not differ between different number of biopsy cells groups (≥10 and <10). The association between number of biopsy cells and GA/BW was also not statistically significant (spearman rank correlation coefficient: $r = 0.014$, $P = 0.911$/$r = -0.025$, $P = 0.843$).
Limitations, reasons for caution: Our study is limited to the retrospective design and small sample size. There was no information available on normal control from natural conceptions.
Wider implications of the findings: Our study offers some insights into the safety of blastocyst biopsy. There were no significant differences in the neonatal status between the biopsy and non-biopsy groups.
Trial registration number: NA

P-572 Perception of pain during the follicular puncture and post-operative (48 h) under paracervical block and conscious sedation, a prospective study

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Study question: Study the level of pain perceived by patients during and 48 h after the oocyte retrieval under local anesthesia and sedation, a prospective study
Summary answer: 83.3% of women reported a high degree of acceptability and satisfaction associated with this method during the oocyte retrieval
What is known already: There are different types of anesthesia that may be used for transvaginal follicular aspiration and oocyte retrieval. Conscious sedation is the most commonly used in IVF because it is relatively safe and does not require the presence of an anesthesiologist when opioids or benzodiazepines are used. Propofol is the preferred anesthetic agent, but should be used by specially trained personnel.

It is easy to administer in cooperative and motivated patients and is safe in healthy individuals; it has a relatively low risk for adverse effects on oocytes, embryo quality and pregnancy rates.

Study design, size, duration: A prospective cohort study of 250 patients. From October 2014 to March 2015, 239 patients answered anonymously the questionnaire, 12 declined and 1 was lost. The study was not performed when oocyte retrieval was done under general anesthesia.
Participants/materials, setting, methods: An anonymous survey was proposed to all patients undergoing IVF/ICSI cycles.

To catalog pain level an scale (0–10) is used, the cut is set to 7 (<7 tolerable pain, intolerable pain ≥7)

To compare the different variables with pain (<7 and ≥7) chi-square test was used and to compare the immediate and postoperative pain nonparametric McNemar test was applied.
Main results and the role of chance: Mean age of patients was 35.4 ± 3 and sterility time was 4.3 ± 2 years. From all the patients 52.8% were from first cycle, and from the ones which had previous cycles in 29% was with general anesthesia

83.3% of women reported a high degree of acceptability and satisfaction associated with this method during oocyte retrieval and 75.3% in the postoperative.
During the oocyte retrieval, the high level of pain was compared in patients who had previous abdominal surgery, gynecological surgery, endometriosis, psychiatric illness, nervous personality, habitual use of analgesics, dysmenorrhea, previous children, compared to patients who did not.

High levels of pain were found in patients with endometriosis (31.3%) comparing to patients with no endometriosis (13.8%), ($p = 0.013$). Also patients with habitual use of analgesics showed higher levels of pain (28.5%) that the ones that do not take analgesics (12.6%) ($p = 0.005$)

Relating to **Postoperative pain** no statistical significance was not found when comparing the same parameters. 52% of the patients who had high pain level in the oocyte retrieval, continued to have it in the postoperative while 23% of patients who had low pain level began to have high level of pain in the postoperative.
Limitations, reasons for caution: it would be interesting to know how many patients have postoperative pain when general anesthesia is performed
Wider implications of the findings: High levels of women´s satisfaction were reported from conscious sedation and paracervical block. However general anesthesia should be offered in patients with endometriosis and in analgesics users. It`s necessary a good analgesia guideline in postoperative pain control.
Trial registration number: CEIC E14/51

P-573 Letrozole use for infertility and the risk of congenital malformations: a systematic review and meta-analysis

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Study question: Does the use of letrozole (LTZ) in infertility increase the risk of congenital malformations relative to clomiphene citrate (CC)?
Summary answer: When used for ovarian stimulation in polycystic ovary syndrome (PCOS) patients and for unexplained infertility, LTZ does not increase the risk of birth defects.
What is known already: LTZ is used as an off-label drug for ovulation induction in PCOS patients as an alternative to CC, and it has been associated with higher ovulation rates and higher live birth rates (LBR) when patients' BMI is >30. In addition, LTZ is used for ovarian stimulation coupled with intra uterine insemination (IUI) for unexplained infertility with equivalent results to CC in terms of LBR. However, LTZ remains contraindicated for use in infertility by many world health authorities because of its potential teratogenic risk. But whether LTZ truly increases the risk of birth defects remains a matter of debate.
Study design, size, duration: A systematic review and meta-analysis of observational studies (OS) and randomized controlled trials (RCTs) on LTZ and the risk of congenital anomalies published before February 2016 was performed. Only studies reporting congenital malformations with LTZ vs. CC were

included. The clinical outcomes of interest included in the meta-analysis are congenital malformations rates. 1015 live births following treatment with LTZ were compared to 1120 live births after treatment with CC.

Participants/materials, setting, methods: We searched Medline, Cochrane Central, EMBASE, Scopus, Web of Science, Google Scholar, as well as related articles from relevant authors on the subject. Studies were only eligible if they compared LTZ to CC for infertility and if they reported the number of congenital malformations. The three authors independently screened for eligibility, and assessed the quality of the studies.

Main results and the role of chance: Out of 526 citations identified, 120 articles met initial eligibility criteria and were further analyzed. Of these, only 6 studies met full inclusion criteria, allowing direct comparison of LTZ to CC and reporting congenital malformations. 3 OS and 3 RCTs were included in the meta-analysis. LTZ has been used either for ovulation induction in PCOS patients or coupled with IUI for the treatment of unexplained infertility. Treatment with LTZ is associated with equivalent risk of congenital malformations when compared to CC. The risk is 1.87% in the LTZ group vs. 3.03% in the CC group, with an odds ratio [OR] of 0.68 [95% CI: 0.33 – 1.40].

Limitations, reasons for caution: Primary outcomes in all the reported studies were clinical pregnancy outcomes and not congenital malformations, which may introduce selection bias and may underestimate the true risk of birth defects. Therefore these results must be interpreted with caution for the time being and patients should be counseled accordingly.

Wider implications of the findings: More data reporting congenital malformations after LTZ use are needed. Ideally, future RCTs powered to detect the birth defect rates are needed to resolve this debate. These may further clarify the safety of this medication.

Trial registration number: NA

P-574 Low immunogenicity potential of follitropin delta, a recombinant FSH preparation produced from a human cell line: Results from phase 3 trials (ESTHER-1 and ESTHER-2)

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Study question: To assess the incidence of anti-FSH antibodies in patients undergoing controlled ovarian stimulation with follitropin delta (FE 999049; recombinant FSH preparation produced from the human cell line PER.C6®) in up to three stimulation cycles.

Summary answer: The incidence of treatment-induced anti-FSH antibodies with follitropin delta was similar to the incidence of pre-existing anti-FSH antibodies and did not increase with repeated exposure.

What is known already: Several factors can influence the immunogenicity of therapeutic proteins, including molecular structure, product purity and formulation, duration of treatment and route of administration. The assessment of antibodies to gonadotropins is not routine in clinical practice, and there are no adequate epidemiological data on the prevalence of antibodies towards FSH in the general population.

Study design, size, duration: Randomised, controlled, assessor-blind trial in patients undergoing controlled ovarian stimulation with follitropin delta or follitropin alfa for IVF/ICSI (ESTHER-1), with a subset of patients undergoing additional assessor-blinded stimulation cycles (ESTHER-2): a total of 1,326 patients in cycle 1, 513 in cycle 2, and 188 in cycle 3. The distribution of patients treated with follitropin delta and follitropin alfa was balanced in all three cycles. Patients with pre-existing or treatment-induced anti-FSH antibodies could be re-exposed.

Participants/materials, setting, methods: In all treatment cycles, blood samples for anti-FSH antibody analyses were collected at stimulation day 1 (pre-dosing) as well as 7–10 days (first post-dosing) and 21–28 days (second post-dosing) after the last gonadotropin dose. The testing strategy was tiered: a screening assay followed by a confirmatory assay and, if applicable, titre, cross-reactivity and neutralising antibody assays.

Main results and the role of chance: The incidence of anti-FSH antibodies prior to exposure to follitropin delta, i.e., pre-existing anti-FSH antibodies, was 1.4% (9/665). After treatment with follitropin delta, the incidence of anti-FSH antibodies was 1.1% (7/665) in cycle 1, 0.8% (2/252) in cycle 2 and 1.1% (1/95) in cycle 3, indicating no increase in anti-FSH antibodies during repeated exposure to follitropin delta. Furthermore, these incidences were similar to those observed for follitropin alfa. In cycle 1, the anti-FSH antibodies observed after treatment with follitropin delta had maximum titres below those detected in patients with pre-existing anti-FSH antibodies. In cycles 2 and 3, all positive anti-FSH antibody samples after treatment with follitropin delta had titres below the limit of quantification. No treatment-induced anti-FSH antibodies in follitropin delta patients were of neutralising capacity (0% in all 3 cycles). Interestingly, repeated treatment with follitropin delta in patients with pre-existing or treatment-induced anti-FSH antibodies did not increase the titre, was not associated with decreased ovarian response, and did not induce immune-related adverse events.

Limitations, reasons for caution: Immunogenicity testing was performed in accordance with current guidelines from the European Medicines Agency (EMA) and the Food and Drug Administration (FDA). All assay cut-points were derived statistically and designed to minimise the risk of false negatives, but a low incidence of false positives cannot be ruled out.

Wider implications of the findings: There is no increased immunogenicity risk following controlled ovarian stimulation with follitropin delta, a recombinant FSH preparation from the human cell line PER.C6®, neither in the first treatment cycle nor after exposure in repeated cycles. The findings indicate an overall low immunogenicity potential of follitropin delta.

Trial registration number: NCT01956110 and NCT01956123

P-575 Birthweight according to hypertensive disorders in pregnancy and conception method – exploring the excess risk of large babies and hypertension following cryopreservation of embryo

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Study question: Is the increased risk of hypertensive disorders in pregnancies following embryo cryopreservation associated with the increased risk of large-for-gestational-age (LGA) offspring after cryopreservation?

Summary answer: The risk of LGA offspring is higher in cryopreservation pregnancies with hypertensive disorders compared to both normotensive cryopreservation pregnancies and spontaneously conceived (SC) normotensive pregnancies.

What is known already: Pregnancies following assisted reproductive technology (ART) carry a higher risk of hypertensive disorders and small-for-gestational-age (SGA) offspring compared to SC pregnancies. More recently, ART pregnancies following cryopreservation have been shown to carry a higher risk of both hypertensive disorders and LGA offspring, compared to both SC and fresh ART pregnancies. Although the causes remain unknown, sibling studies indicate that these excess risks after cryopreservation may not entirely be attributed to parental characteristics or infertility per se.

Study design, size, duration: Population based cohort study with nationwide data from health registers in Sweden, Denmark and Norway from 1988 to 2007. All registered ART singleton pregnancies with gestational age ≥22 weeks

(n = 47,088) and a sample of SC pregnancies (n = 268,599), matched on parity and birth year, were included.
Participants/materials, setting, methods: We compared birthweight in 255,925 normotensive and 12,674 hypertensive SC pregnancies, 37,640 normotensive and 2282 hypertensive fresh ART pregnancies, and 6044 normotensive and 455 hypertensive frozen-thawed ART pregnancies. We used linear regression to compare mean birthweight and logistic regression to compare odds of SGA and LGA outcomes (-/+ 22% according to Marsal's formulas). All analyses were adjusted for parity, maternal age, country, child sex, birth year and gestational age.
Main results and the role of chance: Mean birthweight was lower in SC (−91 g, 95% confidence interval (CI) 100 g to −82 g) and fresh ART (−105 g, 95% CI −126 to −83 g) pregnancies with hypertensive disorders compared to normotensive SC pregnancies. In hypertensive pregnancies following frozen-thawed ART, mean birthweight was 18 g lower than in normotensive SC pregnancies (95% CI −66 g to 29 g). In SC normotensive pregnancies, the risk of being born SGA was 3.9%, whereas the risk was higher in all groups of hypertensive pregnancies, with hypertensive fresh ART pregnancies showing the highest absolute risk of 15.0% (OR 4.4, 95% CI 3.9–4.9). The risk of being born LGA was higher in hypertensive pregnancies, regardless of conception method, but highest in hypertensive pregnancies after cryopreservation with an absolute risk of 8.0% compared to 4.0% in SC normotensive pregnancies (OR 2.1, 95% CI 1.5–3.0). In normotensive pregnancies after cryopreservation, the risk of being LGA was 5.6% (OR 1.4, 95% CI 1.3–1.6).
Limitations, reasons for caution: The number of cryopreservation pregnancies with hypertensive disorders was limited. Residual confounding from parental factors cannot be excluded. We had no information on specific cryopreservation procedures or additional treatment.
Wider implications of the findings: The link between hypertensive disorders and LGA offspring in pregnancies after cryopreservation raises the question of a common underlying pathophysiology for these two complications in frozen-thawed cycles. Improved understanding may open opportunities for prevention.
Trial registration number: Observational study, no trial registration number.

P-576 Ongoing and cumulative pregnancy rates after cleavage stage embryo transfer: impact of the day of cryopreservation of the supernumerary embryos

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Study question: What is the ongoing cumulative pregnancy rate after day 3 transfer in combination with either day 5 vitrification or day 3 slow freezing of supernumerary embryos?
Summary answer: The cumulative ongoing pregnancy rate of day 3 transfers in combination with vitrification is higher than day 3 transfer cycles in combination with slow freezing.
What is known already: A Cochrane analysis demonstrated that in terms of live birth rates there was a slight benefit for fresh blastocyst transfer as compared to day 3 transfer. The cumulative live birth rate showed a benefit for day 3 transfer. However, most of the studies used slow freezing for blastocyst cryopreservation and these protocols proved to be suboptimal. With the introduction of vitrification of blastocysts, better results are obtained in terms of live births It is hypothesized that vitrification at the blastocyst stage might not only increase the cumulative live birth rate after day 5 but also after day 3 fresh transfers.
Study design, size, duration: Retrospective analysis over a period between July 1st 2010 and December 31st 2014 in a group of patients having between 5 and 9 zygotes available on day 1 and with fresh embryo transfer on day 3 (n = 1102). The cumulative ongoing pregnancy rate was defined as the ongoing pregnancy rate obtained after fresh transfer on day 3 in combination with maximum 3 subsequent thawing/warming cycles.
Participants/materials, setting, methods: In 433 patients, the surplus embryos were frozen on day 3 with a slow freezing protocol (Cook, Australia) (group 1) and in 669 patients, the surplus embryos were vitrified at the blastocyst stage (Irvine, USA) (group 2). Continuous variables were compared using the independent Student's t-test and categorical variables were compared using Chi-Square test. The significance level was set at 5% ($p < 0.05$).
Main results and the role of chance: In group 1 and 2, mean female age (32.0 years ± 4.6 years vs. 31.9 years ± 4.7 years; NS) and number of zygotes obtained (6.9 ± 1.4 vs. 6.8 ± 1.4; NS) was similar. The mean number of embryos frozen was significantly higher in group 1 compared with group 2 (2.8 ± 1.5 vs. 2.3 ± 1.3) ($p < 0.0001$). The ongoing pregnancy rates after fresh day 3 transfer were comparable in both groups (27.7% (120/433) for group 1 and 29.0% (194/669) for group 2; NS). The transfer rate after thawing/warming was significantly lower in group 1 (63.6% (246/387) as compared to group 2 (86.3% (550/637) ($p < 0.0001$). The ongoing pregnancy rates after a maximum of 3 consecutive thawing/warming cycles was 15.5% (60/387) in group 1 and 24.3% (153/637) in group 2 ($p = 0007$). The cumulative ongoing pregnancy rate in group 1 was significantly lower (41.6% (180/433) as compared to group 2 (52.2% (347/669) ($p < 0.0001$). Interestingly, the number of patients with an ongoing pregnancy after the first thawing/warming cycle was significantly higher in group 2 (19.0% (121/637) as compared to group 1 (10.3% (40/387) ($p = 0.0002$).
Limitations, reasons for caution: This study is limited by its retrospective design. Our results are conditioned by the lower survival rate of slow freezing of day 3 embryos in group 1.
Wider implications of the findings: These findings suggest that for patients with 5–9 fertilized oocytes, the time to pregnancy can be shortened when the surplus embryos are vitrified on day 5 compared to slowly frozen on day 3. Furthermore, a fresh blastocyst transfer can be suggested whenever there are at least 5 zygotes available.
Trial registration number: Not applicable

P-577 Evaluation of congenital anomalies in 3747 children born after *In Vitro* fertilization (IVF), intracytoplasmic sperm injection (ICSI) or frozen embryo transfer (FET) in France

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Study question: Is the prevalence of congenital anomalies comparable in the three procedures of Assisted Reproduction Technologies (ART) routinely used in our center?
Summary answer: The prevalence of the congenital anomalies is not different when comparing children born after IVF, ICSI and FET.
What is known already: Congenital anomalies (≈2.6% in EUROCAT Database) are one of the main criteria used for assessing children health. The risk for these children to develop such anomalies may vary with factors such as the cause of infertility and different aspects possibly related with Assisted Reproductive Technologies procedure such as ovarian stimulation treatment, culture media composition, embryo culture duration, freezing and thawing cycles and hormonal environment at the time of implantation. It was recently described that subfertile couples are more likely to give birth to children showing abdominal wall defects, hypospadias, right ventricular outflow tract obstruction, and methylation defects causing imprinting disorders.
Study design, size, duration: This study is a retrospective descriptive analysis of congenital anomalies in 3747 children born after IVF, ICSI or FET between 1994 and 2014. Three groups were analyzed: IVF group (1319 children), ICSI group (1563 children) and FET group (575 children).
Participants/materials, setting, methods: Data compiled of congenital anomalies were collected by maternity hospitalization report, a copy of the child health record or an auto-questionnaire filled by parents. Congenital anomalies were coded by a medical doctor using International Classification of Diseases and classified with European Concerted Action on Congenital Anomalies and Twins classification. Variables were described by proportions N(%) and means (SD). Student's t-test was used to compare quantitative variables and Pearson-Chi-Square or Fisher's Exact Test was used for qualitative variables.
Main results and the role of chance: 3457 children were enrolled in the analysis, 1698 boys, 1758 girls and 1 undetermined (2429 singleton, 995 twins and 33 triplets). 290 children were excluded because data could not be verified by a medical report. 142 children [4.1%] were diagnosed with a congenital

anomaly. There are no statistical difference in congenital anomalies prevalence [$p = 0.7$] between the three groups (IVF group: $n = 54$ [4.1%]; ICSI group: $n = 68$ [4.4%]; FET group: $n = 20$ [3.5%]), and between girls ($n = 62$ [3.5%]) or boys ($n = 79$ [4.7%]) [$p = 0.1$]. 3.0% of the children have a congenital anomaly and 1.1% children have more than one. The five main categories of congenital anomalies in our study were: congenital heart defects with 44 cases (1.3%), limb defects with 30 cases (0.9%), genital malformations with 29 cases (0.8%), urinary malformations with 27 cases (0.8%), and 15 cases (0.4%) in the class of genetic syndromes, microdeletion and sequences. Do not report any case of imprinting disease.

Limitations, reasons for caution: This descriptive study does not permit to establish a causal link between these three ART procedures and the congenital anomalies prevalence. However our preliminary results are in line with the epidemiological studies which have shown an increased genetic risk in infertile couples.

Wider implications of the findings: The evaluation of congenital anomalies of children born after IVF, ICSI and FET can be used as a support to assess informed consent to couples enrolled in an infertility treatment program.

Trial registration number: None

P-578 Cortisol levels and diurnal patterns of 9–10 year old children born after ART (IVF/ICSI)

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Study question: Do children born after assisted reproductive techniques (ART) differ from naturally conceived (NC) controls in the levels and diurnal patterns of cortisol at 9–10 years?

Summary answer: ART children showed lower levels of cortisol, particularly in the morning and late afternoon. Diurnal patterns, in contrast, were similar between ART and NC children.

What is known already: Balanced functioning of the HPA system is crucial for stress adaptation, vitality and health. A number of risk factors can lead into altered HPA axis development and cortisol dysregulation, the pre- and perinatal period being especially vulnerable. Even very early events during embryogenesis can affect the development of the HPA system, e.g., through subtle epigenetic changes. The dysregulation of HPA axis can be determined by either altered *levels* or *diurnal patterns* of cortisol. Although pregnancies after ART show increased obstetric and perinatal problems, we have no previous knowledge about HPA axis development and cortisol regulation among ART children.

Study design, size, duration: The prospective follow-up study compares salivary cortisol levels and their diurnal patterns between 9- and 10-year-old ART children ($n = 30$) and their matched pairs of NC children ($n = 30$). The pairs were matched for parental age and education level and child's gender. Statistical analyses further controlled for mother's parity, child's gestational age, birthweight and age at the time of the cortisol assessment.

Participants/materials, setting, methods: Children's cortisol patterns were measured from saliva samples through 5 within-one-day-assessments (awakening, 1 h after awakening, midday, late afternoon and evening). Cortisol level was analyzed using a sensitive method of liquid chromatography-tandem mass spectrometry (LC-MS/MS). Statistical methods included paired *t*-tests and linear mixed-effects modelling.

Main results and the role of chance: Compared to NC children, children born after ART showed lower level of cortisol in the morning right after awakening, ART group $M = 4.75$ µg/l, NC group $M = 6.32$ µg/l, $t(56) = -2.09$, $p < 0.05$, as well as in late afternoon, ART group $M = 1.23$ µg/l, NC group $M = 2.21$ µg/l, $t(56) = -2.48$, $p < 0.05$. In contrast, cortisol diurnal patterns, including awakening response and diurnal decline, were unaffected by assisted reproductive treatment.

Limitations, reasons for caution: Although day-to-day variation in cortisol secretion can be marked, we were unable to collect samples across several days. Our results thus need to be interpreted with caution. Several possible confounders were not included, for example mother-child or father-child relationship quality or children's cortisol levels before middle childhood.

Wider implications of the findings: The findings indicate the importance of considering child endocrine function and HPA system development in understanding the developmental outcomes among children born after ART.

Trial registration number: None.

P-579 Evolution in attitudes towards twin pregnancies in infertile patients. Results in surveys carried out in 2007 and 2015

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Study question: Is the attitude towards twin pregnancies changing in the last years especially in infertile patients receiving information about risks associated multiples pregnancies?

Summary answer: Twin pregnancies continue being a desirable situation for the majority of infertile patients despite receiving information about risks associated with multiple gestations.

What is known already: Multiple pregnancies are considered a serious complication of assisted reproduction technology therefore eSET must be the first option for most patients if not all. One of the biggest obstacles for the implementation of SET policies is the opposition of patients considering twin pregnancies as a desirable situation. Our group and other authors published surveys some years ago finding 75% patients considering twin pregnancies as a positive outcome after IVF. In recent years, efforts have been made to raise awareness about medical and social implications of multiples pregnancies; therefore, to assess changes in attitudes is crucial to design future strategies.

Study design, size, duration: All the women and their partners attending for the first time the reproductive medicine department at Instituto Bernabeu during the period of 4 months from July to December 2015 were offered to fill in a questionnaire. The patients and their partners were required to self-complete the questionnaire separately without consulting each other. To assess changes in attitudes, the questionnaire was identical to the first evaluation carried out in our institution in 2007.

Participants/materials, setting, methods: 275 patients filled the survey (185 women and 90 partners). The questionnaire consists of 15 items valuing the age, sex, level of education, work, previous children, time to conceive, multiple pregnancies in the family or circle of friends. Also we investigated whether they undergone fertility treatments previously. Respondents rated double and triple pregnancies as very negative, negative, indifferent, positive or very positive. The results were compared with an identical survey carried out in 2007.

Main results and the role of chance: 76.0% of respondents considered twin pregnancy as positive or very positive while only 8% saw it as negative or very negative. The results were similar to those obtained in 2007 when 77.9% rated twin pregnancy as positive or very positive compared with 4.9% rated as negative or very negative. Significant differences were observed in terms of the valuation as positive o very positive of triplet pregnancies from 76.0% in 2007 to 49.6% in 2015.

Men and women had similar ratings, 79.3 and 73.1% respectively evaluated twins as positive very positive outcome.

No relationship was found between age and attitude towards twins, average was 36.7 years in patients rating as positive or very positive and 37.0 years as negative or very negative

Patients with children had a more negative attitude to have twins, 54.8 considered it as positive or very positive compared to 79.6% in those without children. Respondents who had received previous IVF treatments and therefore had information about the risks of multiples pregnancies considered twins as more desirable outcome. 85.1% of they rated as a positive or very positive compared with 74.2% in patients without previous treatments.

Limitations, reasons for caution: At present, we receive patients with poorer prognosis (poor ovarian responders and recurrent implantation failure) than in 2007 and this could be a bias factor.

Wider implications of the findings: The strategy to inform patients about neonatal and maternal risks associated with twin pregnancies is useless for changing the attitudes towards twins at least in infertility patients.

Efforts must be done to explain general population and patients that eSET policy is able to improve the success rates per egg retrieval.

Trial registration number: Not a clinical trial

P-580 Calcium dobesilate versus cabergoline for prevention of ovarian hyper stimulation syndrome

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Study question: To compare the effect of oral Calcium dobesilate to oral cabergoline in the prevention of ovarian hyperstimulation syndrome (OHSS) in high-risk women underwent intracytoplasmic sperm injection (ICSI).
Summary answer: Ovarian hyperstimulation syndrome was significantly lower in dobesilate group than in cabergoline group. severe OHSS was more common in cabergoline group
What is known already: Ovarian Hyperstimulation Syndrome (OHSS) is one of the most serious complication of ovulation induction. The pathophysiology of OHSS is characterised by increased capillary permeability, leading to leakage of fluid from the vascular compartment, with third space fluid accumulation. one of the most accepted theory for this is an increase in vascular endothelial growth factor (VEGF).

Cabergoline has been studied as a potential drug for use in the prevention of OHSS due to its role in suppression of (VEGF). Calcium dobesilate is used in the treatment of diabetic retinopathy and chronic venous insufficiency and *in vivo* proved to inhibit VEGF
Study design, size, duration: This was a randomized single blind study in Benha university hospital with two hundred high-risk patients undegoing ICSI or IVF from April 2014 till December 2015 were randomly divided into two groups. The women with even numbers in Group I and Group II with odd numbers. Sample size calculation was

$$SS = \frac{Z^2 \times (p) \times (1 - p)}{C^2}$$

SS = Sample Size.
Z = Z-value.
P = Percentage of population affected.
C = Confidence interval.

Participants/materials, setting, methods: Two hundred high-risk patients undegoing ICSI or IVF characterized by presence of more than 20 follicles by ultrasound, E2 more than 3000 pg/ml or retrieval of more than 15 follicles were randomly divided into 2 groups. The women with even numbers (100) in Group I and were administered calcium dobesilate at day of HCG injection and for 2 weeks while Group II received Cabergoline at day of HCG injection and for eight days.
Main results and the role of chance: One hundred patients in each group completed the study.

Follow-up of the patients showed that OHSS was significantly lower in the calcium dobesilate group (12/100) than cases in cabergoline group (28/100) ($p = 0.005$) and that severe OHSS cases were significantly more common in the cabergoline group (13/100) than in the calcium dobesilate group (2/100) ($P < 0.003$).

All OHSS cases in both groups were early onset OHSS. The patients with mild and moderate OHSS from both the study groups were monitored on an out–patient basis until the resolution of signs and symptoms. All the severe cases in our study were grade 4 of Golan et al. criteria and treated as outpatient. Only one case in the cabergoline group was hospitalized with oliguria (urine output <400 cc per 24 h) and Haemoconcentration (hematocrit >45%).

Fertilization rates, the implantation, chemical and clinical pregnancy and multiple pregnancy rates as well as the number of miscarriages were similar in both groups.
Limitations, reasons for caution: 1- single blind, not double blind study.

2- study no. is a bit small.

3- No control, but this was for ethical reasons as we cannot leave these patients to face the hazards of OHSS.
Wider implications of the findings: Cabegoline showed some success for the prevention of OHSS in the literature with some papers agree and others disagree with its value but for the best of our knowledge this is the first study to try calcium dobesilate as a preventive drug for OHSS and to compare it with cabergoline
Trial registration number: NCT02271360

P-581 The incidence of elevated progesterone levels in modified natural cycle frozen thawed embryo transfer cycles and impact on outcomes

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Study question: What is the incidence of elevated late follicular phase progesterone levels in modified natural cycle frozen thawed embryo transfer (mNC-FET) and what is the effect on clinical pregnancy rates (CPR)?
Summary answer: A progesterone (*P*) level ≥5 nmol/l was observed in 37% of patients undergoing mNC-FET. An elevated progesterone level had no influence on pregnancy rates.
What is known already: Elevated late follicular phase progesterone levels have been associated with lower clinical and ongoing pregnancy rates in patients undergoing fresh embryo transfer after IVF. This has been ascribed to advancement of endometrial maturation resulting in embryo-endometrial asynchrony. It remains unclear to what extent elevated progesterone levels occur in the late follicular phase of mNC-FET and whether they result in a comparable negative effect on pregnancy rates.
Study design, size, duration: The cohort studied in this prospective study derived from the mNC-FET arm of an RCT comparing this means of endometrial preparation with artificial cycle FET (AC-FET). Between February 2009 and April 2014, 969 patients were included in this trial of which 495 were randomized to NC-FET. 271 of these patients were ultimately available for analyses.
Participants/materials, setting, methods: Patients from 17 IVF units were included this study. Ultrasonic monitoring of the dominant follicle was performed until the leading follicle reached a diameter of 16–20 mm. A blood sample was then drawn before administering a 5000 IU dose of human chorion gonadotrophin (hCG). Thawing and transfer was planned based on the moment of hCG injection. The blood samples were centrally analyzed after completion of the trial.
Main results and the role of chance: The ROC curve constructed showed no specific progesterone level above which CPRs were influenced negatively (area under the curve 0.55). 100 of the 271 patients included (36.9%) revealed an elevated serum *P* (≥5 nmol/l) on the day of hCG administration. These patients had a CPR similar to those without a raised *P* level (CPR 25% versus 26%, OR 1.1 95% CI 0.6–2.0). Ongoing pregnancy rate (OPR) did not significantly differ between these two groups (14% versus 15%, OR 1.1 95% CI 0.5–2.4). There was no significant difference in median progesterone level between pregnant and non-pregnant patients (5.1 nmol/l versus 5.0 nmol/l, $p = 0.9$). Logistic regression analyses identified embryo quality as the only independent predictor of clinical pregnancy. A combined LH surge and elevated progesterone in the presence of a dominant follicle on ultrasound (premature luteinisation) occurred in 71 patients (26.2%). There was evidence for a negative trend in CPR in patients with premature luteinisation, however this difference was not statistically significant (CPR 25.5% versus 16.9%, OR 0.6, 95% CI 0.3–1.2).
Limitations, reasons for caution: This study represents a nested subgroup analysis of one arm of an RCT that was designed to answer a different research question. The relatively low OPR limits power for analyzing this secondary endpoint.
Wider implications of the findings: Elevated progesterone levels were commonly observed in mNC-FET cycles but had no apparent impact on pregnancy rates. This suggests that putative causes and impact of raised late follicular phase *P* levels on mNC-FET outcomes differ from those in IVF.
Trial registration number: ANTARCTICA trial from which this cohort derived is registered (NTR number 1586).

P-582 Are criteria for the assessment of blastocyst quality clear enough and embryologists sufficiently trained for reproducibility of blastocyst scoring results?

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Study question: What is the level of reliability/agreement between and within embryologists in assessment of blastocyst quality?

Summary answer: Excellent reliability and agreement in the assessment of blastocyst expansion and overall score. Highest variability in the assessment of trophectoderm and inner cell mass.

What is known already: Numerous blastocyst scoring systems have been developed that take into account blastocoele expansion, morphology of the inner-cell-mass (ICM) and trophectoderm (TE). However, the characteristics of different grades of ICM and TE are generally poorly described. The ICM usually shows substantial inter- and intravariations in size, shape, cell number and cellular connections with TE that depend on blastocyst expansion rate. The assessment of TE is also difficult, especially when fragments or blastomeres are located in subzonal place or within blastocoel. The interobserver variability manifests not only the consistency in embryo assessment between embryologists but shows also the quality of evaluation criteria.

Study design, size, duration: Intra- and interobserver agreement analysis between embryologists of one IVF clinic. Our clinical practise is prolonged cultivation and blastocyst vitrification. Evaluation of blastocysts is based on morphological parameters using Gardner's system for assessing ICM, TE and degree of blastocoele expansion and our originally developed scoring system which takes into account blastocyst's implantation ability therefore blastocysts are ranked in eight classes (B1–B8) providing an overall score. This system considers the presence of excluded blastomeres and/or fragments.

Participants/materials, setting, methods: Five experienced embryologists took part in this study. Seventy randomly selected images of day-5 embryos of different morphology, from morula to hatching blastocyst, were presented to each embryologist twice for evaluation in the span of one week. Intraclass correlation coefficient (ICC) was used to calculate the intra- and interobserver agreement and was interpreted as perfect (>0.81), substantial (0.61–0.80), moderate (0.41–0.60), fair (0.21–0.40) and slight (<0.20) (Viera and Garrett, 2005).

Main results and the role of chance: The ICC analysis of interobserver variability showed perfect agreement (reliability of average measures) for the whole group of observers in the assessment of the blastocyst expansion ($ICC_{average}$ = 0.958, 95% CI = 0.940–0.972), morphology of the ICM ($ICC_{average}$ = 0.830, 95% CI = 0.758–0.886), TE (ICC = 0.827, 95% CI = 0.754–0.884) and in blastocyst scoring ($ICC_{average}$ = 0.927, 95% CI = 0.895–0.951). Unfortunately embryo scoring is performed by a single embryologist therefore single measures ICC represents more realistic situation. The reliability of single measures ICC was substantial in the assessment of expansion (ICC_{single}=0.820, 95% CI = 0.759–0.873) and in blastocyst scoring (ICC_{single} = 0.718, 95% CI = 0.631–0.796) and only moderate in the assessment of ICM (ICC_{single} = 0.495, 95% CI = 0.386–0.608) and TE (ICC_{single} = 0.489, 95% CI=0.380–0.603).

In the analysis of intraobserver variability the agreement in repeated measurements ($ICC_{average}$) and the reliability of single measurement (ICC_{single}) of each individual was calculated. There was perfect to substantial agreement for the assessment of blastocyst expansion ($ICC_{average}$ varied between 0.960 and 0.942; ICC_{single} varied between 0.891 and 0.923) and substantial to only moderate agreement for the assessment of other blastocyst quality parameters ($ICC_{average}$ varied between 0.960 and 0.942; ICC_{single} varied between 0.789 and 0.440). The lowest reliability and agreement (substantial to moderate) was noted in the assessment of ICM and TE.

Limitations, reasons for caution: Assessment of embryo morphology from images often results in different interpretations. Blastocyst scoring of ICM and TE is very subjective, reliable only in fully expanded blastocysts. Assessment under microscope or with time-lapse provides more information for monitoring of embryonic development which may improve agreement in embryo assessment.

Wider implications of the findings: Morphological assessment is still the most frequently used criteria for selecting embryos for transfer or cryopreservation. In order to optimize IVF/ICSI treatments it is important that embryos are assessed uniformly which implies an internal and external quality control as crucial elements of modern IVF laboratory.

Trial registration number: This is not a clinical trial.

P-583 Pre-treatment with oral contraceptive pill (OCP) in IVF antagonist cycles: may it affect reproductive outcomes? A retrospective study

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Study question: Ovary and implantation outcomes in patients synchronized with OCP for a short or a long period during IVF antagonist cycles compared with patients that didn't receive the drug.

Summary answer: A statistically significant difference has been observed in the different groups in term of ovary outcomes, implantation, pregnancy, biochemical pregnancy and spontaneous abortion rates.

What is known already: Griesinger in his meta-analysis (2010) found ongoing pregnancy rate significantly lower in patients with OCP pre-treatment during IVF Antagonist GnRh protocol. Velasco, in a randomized controlled trial (2011), found no difference in live birth rate among patients with OCP in antagonist IVF cycles or with a long term protocol. In a recent review Velasco et al. (2015) describes with caution that OCP treatment might have a negative effect on outcome in GnRh antagonist IVF Cycles. On the contrary, Orvieto et al. (2014) believes that OCP pre-treatment have a positive role, especially during a stimulation protocol known as ultra-short flare GnRh-agonist/GnRh-antagonist.

Study design, size, duration: In our retrospective multicentric cohort study we analyzed 5885 GnRH antagonist IVF cycles performed in 4718 patients from January 2002 to July 2015. Three groups have been identified: Group A Group B and Group C, respectively of 187,1689,3049 patients. The average both FSH and HMG dosage administered was comparable in all groups (rFSH 2,195.7 ± 1,231.8 UI HMG 3,126.3 ± 1,968.2 UI) Endometrial tickness on day of embryo transfer was between 9.5 and 10.1 mm in all groups.

Participants/materials, setting, methods: All patients included are between 18 and 37 years old and BMI was ≤35 Kg/m2. Poor Responders was estimated in <2%. Group A and Group B were pre-treated with ethynilestradiol 0.03 mg+ gestoden 0.075 mg. Group A for a period ranging from 7 to 12 days immediately followed by the administration of Gonadotropin. Group B for a period ranging from 13 to 40 day, waiting for menses with gonadotropin.

Main results and the role of chance: Chi-square test among the proportions and ANOVA test for the comparisons among the average multiples, have been used. We found a significant difference (p = 0.002) between Group A vs. Group C and Group B and Group C for the number of follicles (p = 0.001), for MII oocytes and fertilized oocytes (p = 0.002). Implantation rate for Groups A, B and C was 15.8%, 16.9% and 20.0%, respectively. The correlation between the group of treatment has come out as significant (chi2 = 16.3569; p = 0.0003). The pregnancy rate found in Groups A, B and C was of 23.5%, 27.4%, 30.3% respectively. The correlation between the group of treatment has come out as significant (chi2 = 7.5761; p = 0.023). The biochemical pregnancy rate in Groups A, B, C was 11.8%, 8.1 and 6.8% respectively. A significant correlation to the group of treatment has been observed (chi2 = 7.8956; p = 0.019). Abortion rate in Group A was 5.4%, in Group B 6.7% and in Group C 6.2%. The differences observed in the abortion rate were not correlated to the group of treatment. Twinning were found with a similar frequency in the three groups (5.4%, 5.5% and 5.3%) with no statistical significant differences among the groups.

Limitations, reasons for caution: Our data shows a worsening of outcomes in patients pre-treated with OCP. We argue that caution must be exercised in drawing conclusions too quickly on whether or not OCP might have a negative effect. Although a large patient's number, it's necessary a prospective randomized trial to confirm our data.

Wider implications of the findings: We found a significant negative effect of OCP pre-treatment (Group A and Group C), disagreeing with Bellver et al. (2007) that doesn't show significative differences whether or not OCP pre-treatment. The real benefits of OCP c have to be clarified, together with the correct protocols (long or short) during IVF cycles.

Trial registration number: not a clinical trial

P-584 Placental anomalies in singleton pregnancies after assisted reproduction technology: a systematic review and meta-analysis

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Study question: Are singleton pregnancies after assisted reproduction technology (ART) associated with a higher risk of placental anomalies as compared to pregnancies after spontaneous conception (SC)?

Summary answer: Singleton pregnancies after ART are at an increased risk of placenta praevia and placental abruption as compared to spontaneously conceived singleton pregnancies.
What is known already: Placental anomalies (placenta praevia, placental abruption, morbidly adherent placenta and cord-insertion anomalies) in singleton pregnancies are not common but are associated with significant maternal and neonatal morbidity and mortality. It has been suggested that pregnancies after ART represent a high risk group where the incidence of these placenta anomalies is increased. Although, various epidemiological studies have been performed evaluating the obstetric outcomes after ART, a systematic review focused on evaluating the risk of placental anomalies in ART singleton pregnancies when compared with spontaneously conceived pregnancies, is currently lacking.
Study design, size, duration: This is a systematic review and meta-analysis of comparative studies. In November 2015, electronic searches were conducted on medical databases (MEDLNE, EMBASE, Cochrane, CENTRAL, Web of Science, Scorpus, ISI) for studies meeting predefined eligibility criteria (from 1978 to present). Data was extracted and methodological quality assessed independently by two reviewers. Studies to be included were appraised for risk of bias and suitability for meta-analysis. Subgroup analysis allowed for the identification of heterogeneity moderators.
Participants/materials, setting, methods: As eligible were considered observational studies examining the occurrence of placental anomalies in singleton pregnancies following ART compared to that of spontaneously conceived singleton pregnancies. Outcomes examined were the incidence of placenta praevia, placental abruption, placenta accreta/increta/percreta, as well as anatomical or cord insertion anomalies. Overall, *30* studies were included in the current meta-analysis, representing a sample of *5*8,6*98* ART pregnancies compared to a cohort of 3,9*2*8,3*01* spontaneously conceived pregnancies.
Main results and the role of chance: The incidence of placenta praevia in singleton pregnancies after ART was significantly increased as compared to pregnancies spontaneously conceived (OR: OR: 3.56, 95% CI: 2.80–4.52; $n = 25$ studies). This finding was confirmed by limiting the analysis to matched studies or studies providing estimates adjusted for various confounders ($n = 20$) confirmed this finding (OR: 2.90, 95% CI: 2.34–3.75).

Placental abruption also appeared to be significantly more frequent in singleton pregnancies after ART as compared to singleton pregnancies after spontaneous conception (OR: 1.91, 95% CI: 1.67–2.19; $n = 16$ studies). This finding was confirmed by limiting the analysis to matched studies or studies providing estimates adjusted for various confounders ($n = 11$) confirmed the original finding (OR: 1.74, 95% CI: 1.48–2.05).

Abnormal cord insertion was evaluated in only three studies, the statistical synthesis of which suggested a significantly increased incidence of cord insertion anomalies in singleton pregnancies after ART as compared to pregnancies after spontaneous conception (OR: 3.07, 95% CI: 1.59–5.95).

A single eligible study suggested the presence of increased risk for placenta accreta in singleton ART pregnancies as compared to the spontaneously conceived ones (OR: 2.67, 95% CI: 1.42–5.02).

No significant difference was detected in terms of abnormal placental shape between ART and SC singleton pregnancies (OR: 1.72, 95% CI: 0.19–15.28)
Limitations, reasons for caution: Limitations relevant to this meta-analysis may arise from terminology discrepancies on behalf of the included studies. Although, adjusted estimates were used, the presence of residual confounding factors cannot be excluded. This meta-analysis was not designed to distinguish between the effect of ART use and that of underlying infertility.
Wider implications of the findings: The widespread use of ART necessitates an appreciation of the maternal health outcome and risk stratification. This meta-analysis offers a comprehensive assessment of the placental-related obstetric risk for appropriate counseling of patients when undergoing ART.
Trial registration number: Not applicable

P-585 The birth weight of term singletons after frozen-thawed embryo transfer (FET) treatments is higher using hormonal substitution than natural cycle

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Study question: Is the protocol for preparation of the endometrium in frozen embryo transfer (FET) treatment related to the birth weight at term?
Summary answer: Singleton term newborns after FET hormonal substitution are independently and significantly heavier than children born after FET natural cycles
What is known already: FET treatments are increasingly important in fertility treatments. Studies suggest a higher live birth rate and heavier newborns after the transfer of frozen as compared to fresh embryos. This might be due to improved laboratorial conditions or less disturbed peri-implantation environment in frozen cycles. FET, in combination with GnRHa-trigger can overcome ovarian hyperstimulation syndrome, high estradiol or high progesterone levels seen in some fresh cycles. The gold standard protocols of FET are the hormonal substitution or the natural cycle protocols. Studies suggest that hormonal substitution may lead to a higher early pregnancy loss rate in comparison to natural cycle.
Study design, size, duration: We have retrospectively collect all frozen embryo transfers from 4 central hospitals and 2 private centers, that resulted in 2428 term singleton newborns, from 1997 to 2015. From those cycles, 1022 (42%) were performed in spontaneous cycle with progesterone support during the luteal phase, and 1406 (58%) in hormonally substituted cycles (E2 + *P*).
Participants/materials, setting, methods: We compared the birth weight of the newborns at term after FET cycles in natural cycles with the newborns after FET in substitution cycles. After uni-factorial analysis, we built up a multivariate logistic regression analysis to identify the factors that independently influenced the weight of the newborns
Main results and the role of chance: During the study period, the percentage of preterm births was similar in fresh (8.3%) and in frozen cycles (7.4%). The delivery of multiples, as well as deliveries of singletons born pre-term or post-term or from mothers with a BMI over 35, were excluded from further analysis.

The mean birth weight of singleton term children conceived after FET treatment using hormonal substitution was significantly higher ($p < 0.0001$) than that of children conceived after natural cycle FET (3675 ± 488 vs. 3563 ± 486 gr, respectively). This effect was noted both in male and female newborns. A multifactorial regression model analysis identified the protocol of treatment ($p < 0.0001$), the gender of the newborn ($p < 0.0001$), the gestational age ($p < 0.0001$) and the method of fertilization ($p < 0.016$) as independent factors for the birth weight. The highest mean birth weight occurred in boys born after FET treatments with hormonal substitution and fertilization with ICSI technique.
Limitations, reasons for caution: The study is retrospective and includes treatments from many IVF centers. However, the sample size is large. Other unknown factors, not identified in the logistic regression model, might influence the birth weight
Wider implications of the findings: The identification of various factors during FET treatment, which may influence the outcome of the newborns are of outmost importance. In this aspect the hormonal substitution protocol might have an important impact on epigenetic factors, peri-implantation, and early placentation, which influence the birth weight of the newborn
Trial registration number: None

P-586 Genome-wide DNA methylation evaluation of cord blood from assisted reproductive technology (ART) neonates

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Study question: To determine whether sperm injection and/or in-vitro culturing of embryos alters the DNA methylation in the cord blood of neonates.
Summary answer: The DNA methylation in the cord blood of ART neonates appears to be comparable to that of naturally conceived (NC) neonates.

What is known already: ART procedures such as sperm injection into oocytes during intracytoplasmic sperm injection (ICSI) and in-vitro culturing of embryos during in-vitro fertilization (IVF) have been suggested to alter the DNA methylation in the cord blood of neonates. However, previous studies have not included a proper control for the underlying infertility of the ART population. There is yet to be a comparative DNA methylation study including neonates born from intrauterine insemination (IUI), which is a treatment for sub-fertile couples and includes ovulation induction and sperm processing, but not in-vitro conditions.
Study design, size, duration: A control-treatment study including 15 ICSI, 17 IVF, 16 IUI, and 16 NC singleton neonates. Written consent and questionnaires were obtained from the parents prior to birth. Cord blood samples were collected at birth. This study was approved by the UBC research ethics board.
Participants/materials, setting, methods: DNA was extracted from cord blood and bisulfite converted. Samples applied to the Illumina GoldenGate Methylation Cancer Panel I for the evaluation of *1,5*05 CpG sites. Microarray results were analyzed in Illumina BeadStudio (3.1.3.0). Primers were designed to target regions incorporating candidate microarray CpGs using the PyroMark Assay Design 2.0. Pyrosequencing was used to analyze the DNA methylation of these regions using the PyroMark Q96 MD. Statistical analyses were conducted in *R* (3.2.3).
Main results and the role of chance: We previously showed that altered DNA methylation at CpG sites were found (via microarray) in the cord blood of IVF neonates when compared to both IUI and NC, but not ICSI neonates. The RARRES1 promoter was selected for DNA methylation verification via pyrosequencing due to high statistical significance and greatest absolute DNA methylation difference between IVF and IUI (+16.3% in IVF; $P < 0.001$). RARRES1 promoter DNA methylation alterations have been associated with cancer; however, no studies have looked at this promoter with regards to reproduction and development. A total of 6 CpG sites within the promoter, including cg12199224 which was on the microarray, were evaluated for DNA methylation in 5 ICSI, 9 IVF, 6 IUI, and 9 NC neonates. The mean DNA methylation over the region, as well as at individual CpG sites, was not significantly different between groups (ICSI = 5.1% ± 1.4%, IVF = 4.1% ± 1.8%, IUI = 4.0% ± 1.1%, NC = 5.0% ± 2.8%).
Limitations, reasons for caution: The study evaluated a limited number of CpG sites within the promoter region of the RARRES1 gene. In addition, not every case has yet been verified by pyrosequencing. A larger sample size and verification of other candidate CpGs/regions are further needed to confirm the previous microarray results.
Wider implications of the findings: Our preliminary findings suggest that IUI neonatal cord blood may not be significantly different in DNA methylation compared to those from ART. This may suggest that ART procedures such as sperm injection and/or in-vitro culturing may not be significantly altering the DNA methylation in neonates.
Trial registration number: N/A

P-587 DNA methylation and gene expression of imprinted genes is not associated with altered placental-fetal weight ratio in singleton births

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Study question: To determine whether pregnancies with higher placental-fetal weight ratio (PW:FW) have altered DNA methylation and/or gene expression at imprinted genes.
Summary answer: There were no differences in DNA methylation and gene expression in the cord blood or placental tissue between high PW:FW ratio and normal ratio pregnancies.
What is known already: Assisted reproductive technology (ART) pregnancies have been previously associated with altered DNA methylation and gene expression at imprinted genes. Furthermore, ART pregnancies have a higher incidence of high PW:FW ratio as compared to naturally conceived (NC) pregnancies. However, whether the subset of pregnancies with high PW:FW ratio have altered DNA methylation and gene expression at imprinted genes is not well studied.
Study design, size, duration: A control-treatment study using data collected between 2003 and 2015 on 375 singleton births (67 ICSI, 11 IUI, 63 IVF, 234 NC) from Vancouver. Births from pregnancies complicated by smoking, medications during pregnancy, gestational diabetes, hypertension, pre-eclampsia, placental previa, preterm, and intrauterine growth restriction were not included.
Participants/materials, setting, methods: Placenta and cord blood was obtained at birth from pregnancies who gave written consent. The PW:FW ratio were calculated from the placental weight over the birth weight. Cord blood and placental tissue were extracted for DNA and RNA. DNA methylation was evaluated by pyrosequencing at the imprinted genes KvDMR1, PEG10, PLAGL1, and LINE-1. Gene expression was studied by RT-qPCR at the imprinted genes CDKN1C, IGF2, KCNQ10T1, and PLAGL1.
Main results and the role of chance: We found a significantly increased PW:FW in IVF vs. NC ($p < 0.0001$) as well as ICSI vs. NC ($p < 0.005$). We also found a significantly increased placental weight in IVF vs. NC ($p < 0.0001$) and ICSI vs. NC ($p = 0.018$). We saw no significant difference in fetal weight in IVF vs. NC ($p = 0.700$) and ICSI vs. NC ($p = 0.872$). All these differences remained significant or very nearly significant regardless of the exclusion of preterm, IUGR, or SGA births. Among births that were at least two standard deviations greater than the mean PW:FW of NC births ($n = 19$), we found no significant differences in the expression of CDKN1C, IGF2, KCNQ10T1, and PLAGL1 mRNA compared to all other births. Similarly, DNA methylation in KVDMR1, LINE-1, PEG10, PLAGL1 was not found to be significantly different between groups.
Limitations, reasons for caution: The number of cases with higher PW:FW ratio was limited. Not all cases had complete clinical information for potential confounding factors. Furthermore, not all cases were included in the DNA methylation and gene expression analyses.
Wider implications of the findings: The altered DNA methylation and gene expression seen among ART births in previous studies may not be entirely responsible for the increased PW:FW. Alternatively, altered DNA methylation and gene expression among ART cohorts in previous studies may not be significantly influenced to the small subset of high PW:FW ratio pregnancies.
Trial registration number: N/A

POSTER VIEWING SESSION

REPRODUCTIVE (EPI)GENETICS

P-588 Genetic polymorphism in the vitamin D receptor gene and 25-hydroxyvitamin D serum levels in east Indian women with polycystic ovary syndrome

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Study question: Whether the VDR gene polymorphisms are associated with susceptibility to PCOS.
Summary answer: The present findings suggest that the Apa-I, Fok-I polymorphism of the VDR gene is associated with PCOS and seems to modulate ovarian steroid secretion.
What is known already: Polycystic ovary syndrome (PCOS) is the most common metabolic abnormality such as changes in lipid profile, diabetes, hypertension and metabolic syndrome occurring in young women of reproductive age. Low vitamin D levels were found to be associated with the development of obesity and insulin resistance in women with PCOS. Variants on vitamin D receptor (VDR) gene have also been related to metabolic comorbidities in general population.
Study design, size, duration: A case-control study was done.125 women with PCOS were enrolled, along with 82 women without any evidence of PCOS as a control group; the women of both groups were aged 16–40 years.

Participants/materials, setting, methods: Women with PCOS and a control group, all aged 16–40 years, were enrolled. Genotyping of VDRFok-I (rs2228570), VDR Apa-I (rs7975232) as well as GC (rs2282679), DHCR7 (rs12785878) SNPs between groups were determined by using direct sequencing. Serum 25-hydroxyvitamin D [25(OH)] levels were measured by ELISA.
Main results and the role of chance: Mean serum 25(OH)D in the PCOS and control samples were 19.08 ± 7 and 23.27 ± 6.03 ($p = 0.048$) which were significantly lower in PCOS patients compared with controls. CC genotype of the VDR Apa-I SNP was same frequent in PCOS (25.6%) and controls (25.6%) (OR: 0.9995; 95% CI: 0.528 to 1.8921; $p = 0.9987$). The CC genotype was also significantly associated with lower E2 ($p = 0.031$) and androstenedione levels ($p = 0.026$). We observed a significant association of GC polymorphism with 25(OH)D levels. PCOS women carrying the GG genotype (in GC genes) had significantly higher risk for vitamin D deficiency than women carrying the TT genotype.
Limitations, reasons for caution: A limitation of the study is the small sample size; therefore, we have to be careful in making this conclusion.
Wider implications of the findings: Polymorphisms of the VDR gene might be associated with PCOS and biochemical markers related to PCOS.
Trial registration number: ECR/130;67/Inst/WB/2013

P-589 Comparison clinical outcome of next generation sequencing (NGS), aCGH and FISH on pre implantation genetic screening in frozen thaw embryo transfer cycle

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Study question: To determine the efficiency of NGS for pre implantation genetic screening in FET cycle compare with aCGH and FISH from both younger and older patients.
Summary answer: Data show NGS results in high pregnancy rates. NGS and aCGH could be beneficial as the euploid selection and allowing single embryo-transfer without decreasing outcome.
What is known already: A variety of methodologies for 24 chromosome analysis have been develop and available for clinical use. Currently used PGS methods of aneuploidy screening such as aCGH have been reported to improve pregnancy rates. The recent advances in NGS have provided new methods for screening embryos. It has shown promise of improved clinical outcome. However, there is still limited information of NGS in clinical setting.
Study design, size, duration: This is retrospective study. 718 patients were enrolled in this study during the period of January to July 2015 and divided into two groups according to female age. One is younger group (<38 years) ($n = 607$: NGS = 299, aCGH = 152, FISH = 42, no PGS = 114) and second is older group (≥38 years) ($n = 111$: NGS = 34, aCGH = 42, FISH = 12, no PGS = 23). Euploid blastocysts were transferred based on the PGS results in FET cycle.
Participants/materials, setting, methods: A total of 4,790 blastocysts were biopsied and then performed FISH, aCGH and NGS according to their studied groups. The method involed whole genome amplification followed by NGS veriseq kit or 24 sure array-CGH kit. Copy number variation analysis was accomplished with BlueFuse Multi software. FISH method including of cell spread on slide, fixed, hybridization and washed according to instruction of multivision PGT for chromosome 13, 18, 21, X and Y probe (Vysis).
Main results and the role of chance: NGS can detect all type of chromosome abnormality including of single (24%), dual (11%), and complex (8%). Interestingly, the percentage of embryos which called mosaicism by NGS is significantly higher than aCGH (NGS = 3.68% vs. aCGH = 1.2%, $p < 0.001$). The euploid rate from older were lower than from younger female (NGS = 26.1 vs. 54.5%, aCGH = 35.3 vs. 59.3% and FISH = 41.4 vs. 52.8%, $p < 0.01$).

Clinical pregnancy rate from NGS is comparable with aCGH but higher than FISH and no PGS in both younger and older female (<38 years old group: NGS = 61.8%, aCGH = 57.1%, FISH = 52.4%, no PGS = 48.2% and ≥38 years old group: NGS = 60.5%, aCGH = 56.6%, FISH = 16.7%, no PGS = 10%). The average number of embryo per transfer from NGS and aCGH groups were significant decrease when compare with FISH and no PGS group in both younger and older female (younger group: NGS = 1.4, aCGH = 1.4, FISH = 1.6, no PGS = 2.1, $p < 0.0001$ and older group: NGS = 1.2, aCGH = 1.2, FISH = 1.8, no PGS = 2.0, $p < 0.0001$).

Additionally, eighty-eight cleavage biopsied embryos which resulted abnormal by FISH were re biopsied on blastocyst stage then performed aCGH or NGS. The percentages of euploid blastocyst after re biopsy are similar for both aCGH and NGS (43.24% vs. 43.14%).
Limitations, reasons for caution: This study show high pregnancy outcomes following transfer euploid blastocyst in frozen thaw cycles. Clinical effectiveness after transfer in fresh cycle should be further study.
Wider implications of the findings: This study report an application of NGS-based comprehensive aneuploidy screening on blastocyst stage in a clinical setting versus aCGH and FISH 5 probe. NGS methodology is representing a valuable alternative to the other comprehensive aneuploidy screening techniques currently available.
Trial registration number: -

P-590 Characterization of promoter of human SET gene

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Study question: What is the promoter sequence and proximal minimal promoter region of SET? Which transcription factors (TFs) may regulate SET expression?
Summary answer: SET core promoter was located within the region −157/+47 relative to the transcription start site. TFs like ZFX, E2F and NRF-1 may regulate its activity.
What is known already: SET is a multifunctional protein involved in histone binding, nucleosome assembly, transcription control and cell apoptosis. Our previous studies showed SET was one of the over-expressed genes in ovaries of patients suffering from polycystic ovary syndrome (PCOS) when compared to normal ovaries using cDNA microarray technology. We also found that in theca cell, SET played a positive role in regulating ovarian androgen biosynthesis by enhancing the transcription of steroidogenic enzymes 3beta-hydroxysteroid dehydrogenase 2 (HSD3B2) and cytochrome P450 17 alpha hydroxylase/lyase (P450c17) and increase lyase activity of P450c17, which maybe lead to the hyperandrogenism in PCOS.
Study design, size, duration: We isolated a genomic clone of the human SET gene promoter containing 998 nucleotides of the 5' flanking region. The sequence was analyzed by blast and UCSC, and then cloned into pGL3-Basic vector to construct SET promoter reporters. Using a series of 5' deletion promoter plasmids in luciferase reporter assays, we identified the core region which is sufficient for full promoter activity. Applying Biology Information Technology, we can then predict the candidate TFs for SET.
Participants/materials, setting, methods: A nearly 1.2 kb 5'-flanking region of SET gene was amplified by polymerase chain reaction and digested with NheI and XhoI, then subcloned into promoter-less luciferase expression plasmid pGL3-Basic. So did the deletion clones. DNA sequence analysis confirmed these sequences. Firefly and Renilla luciferase activities were measured with the Dual-Luciferase Reporter Assay System after the transiently transfection of the reporter plasmids with various lengths. Jarspar and genomatix programs were used to predict TFs of SET.
Main results and the role of chance: HeLa cells and HEK293 cells have similar profiles of luciferase expression upon transfection. The promoter region extending from −996 to +137 showed activity and progressive deletion experiments showed region −157 to +137 having the strongest promoter activity. Moreover, the activity of +47/+137 construct was as low as the empty control plasmid, indicating that the essential regulatory element(s) necessary to sustain the basal transcriptional activity is located within sequences between −157 and +47. This region lacks typical TATA box but contains CCAAT box and a highly GC-rich content. Analyzing the region with the Jaspar and Genomatix websites, we found several DNA-binding sites have high scores in both programs, including E2F1, E2F3, E2F4, ZFX, SP1, and NRF-1 site. They are the candidate TFs which can regulate SET transcription expression. SET is a newly uncovered gene associated with hyperandrogenism in PCOS. Understanding the transcriptional regulation of SET may contribute to therapeutic strategies aimed at altering high androgen level in patients with PCOS and help them to resume normal ovulation.
Limitations, reasons for caution: As a gene associated with androgen synthesis, SET is prominently expressed in ovarian theca cells and oocyte. It's reasonable to conduct experiments in these cells. However, the cell lines of these cells

are unavailable. In the further study, we will culture theca cells to clarify the transcription regulation of SET.
Wider implications of the findings: SET is overexpressed in aggressive cells from malignant neoplasm, and contributes to cell survival. In gynecologic area, SET accumulates in ovarian cancer and breast cancer. Illustration the transcription regulation of SET may lead to the development of novel drugs with improved anti-cancer activity without immune suppression and other toxic effects.
Trial registration number: none

P-591 Karyomapping: a retrospective case series of clinical application of karyomapping for couples requiring preimplantation genetic diagnosis for single gene disorders and/or chromosomal rearrangements

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Study question: Can karyomapping be applied in a clinical setting to couples requiring preimplantation genetic diagnosis (PGD) for single gene disorders and/or chromosomal rearrangements?.
Summary answer: Clinical application of karyomapping offers an efficient and robust method for PGD for single gene disorders and chromosomal rearrangements, with several advantages over conventional methods.
What is known already: PGD is an early form of prenatal diagnosis. Although generally successful, traditional PGD strategies based upon the polymerase chain reaction (PCR) are associated with several technical and logistical limitations. These include allele dropout, which impacts diagnostic accuracy, long work-up times required in order to tailor tests to individual patients, and failure to detect lethal aneuploidies. In contrast, karyomapping represents a more generic approach. The method utilises microarray technology to determine the genotype of >300,000 single nucleotide polymorphisms (SNP). The genetic status of individual embryos can be determined by analysis of linked SNP alleles inherited along with the mutant gene.
Study design, size, duration: This is a retrospective case series of PGD cycles from May 2014 to December 2015. Karyomapping was considered feasible if the DNA from a relative of known genetic status or from an affected foetus was available. Karyomapping was applicable in 90 couples during this period of time. 76/90 (84%) couples had PGD by karyomapping. The remaining 14 couples (16%) are awaiting treatment at the time of writing.
Participants/materials, setting, methods: Couples self-referred or were referred from regional genetic centres. Couples were counselled and standard IVF protocols were applied. Initially, couples had day-three embryo biopsy and transfer of a suitable blastocyst. This strategy was changed in May 2014 to blastocyst biopsy, vitrification and subsequent frozen embryo replacement. The karyomapping was carried out by Reprogenetics, UK, essentially as described by Natesan et al. (2014). Patients were advised to do urinary pregnancy test 16 days after embryo transfer.
Main results and the role of chance: 85/90 (95%) and 3/90 (3%) couples were referred for autosomal single gene disorder and X-linked disease, respectively. The remaining 2/90 (2%) were referred for chromosomal rearrangements. Additional, PCR or array comparative genomic hybridization (a-CGH) was carried out in order to increase diagnostic accuracy in 5/90 (6%) and 1/90 cases, respectively. A total of 84 transvaginal oocyte retrievals and 85 cycles of embryo biopsies were performed. One couple had blastocysts transferred from an IVF centre elsewhere. Day-3 embryo and blastocyst biopsies were carried out in 16/85 (19%, 95% CI 12–28) and 69/85 (81%, 95% CI 72–88) cycles, respectively. A total of 72 embryo transfers were performed. 60/72 (83%, 95% CI 73–90%) and 12/72 (17%, 95% CI 10–27) were frozen and fresh embryo transfers, respectively. From 63 embryo transfers with a known pregnancy outcome, 42/63 (67%, 95% CI 54–77) had a positive pregnancy test. The ongoing pregnancy rate is 37/63 (59%, 95% CI 46–70). 2/63 (3%, 95% CI 1–11) embryo transfers were biochemical pregnancies and 3/63 (5%, 95% CI 2–13) embryo transfers have resulted in early first trimester miscarriages. 21/63 (33%%, 95% CI 23–46) had a negative pregnancy test. In 9/72 (13%, 95% CI 7–22) embryo transfers, the outcome is pending.
Limitations, reasons for caution: This is the largest karyomapping dataset so far reported, but results are retrospective and non-randomized.
Wider implications of the findings: Karyomapping is reliable, accurate and efficient for couples requiring PGD for single gene disorders and/or chromosomal rearrangements. Its utilisation of a generic protocol dramatically accelerates test work-up compared to conventional PCR, helping to expedite the start of treatment. Additionally, it provides aneuploidy screening, minimising risks of miscarriage and implantation failure.
Trial registration number: N/A

P-592 Is the number of previous embryo transfer cancellations a predictor of next cycle outcome in preimplantation genetic screening (PGS) cycles?

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Study question: Is the number of previous PGS cycles without euploid embryo correlated with the likelihood to find at least one euploid embryo in the next PGS?
Summary answer: There is an increase in the chances of having an embryo transfer in a second cycle if in the first there was an euploid embryo
What is known already: PGS has been used in an *In Vitro* Fertilization (IVF) to improve the implantation rate and to reduce the miscarriage rate for a long time. Recently the technology has allowed us to analyze the whole chromosome complement. There are many patients that repeat cycles without a limit. However, little is known about the effect of the previous cancellations of embryo transfers. We should ask ourselves when we should stop
Study design, size, duration: An observational, retrospective, multicenter study of the patients included in the cycles of PGS dated from January 2011 to June 2015 in IVI clinics. A total of 820 patients were included in this study, age ranging from 27 to 48 years. The inclusion criteria were: recurrent miscarriage (≥2 previous abortions), implantation failure (≥3 previous IVF failures), Advanced Maternal Age (≥38 years), male factor ($<5 \times 10^6$/ml)
Participants/materials, setting, methods: All patients underwent PGS and analysis was performed using Array-CGH. All the patients have performed more than one PGS cycle. Patients were divided in different groups taking into account if they had transfer or not in the previous cycles. In order to determine whether the lack of euploid embryo relates to the rate of embryo transfer in the next cycle, cycles with euploid embryos but without embryo transfer, because the embryos were arrested, were excluded
Main results and the role of chance: The likelihood of having embryo transfer in each cycle if the patient had not it in the previous one was: 2nd cycle: 37% (n = 663); 3rd cycle: 55% (n = 134); 4th cycle: 0% (n = 23). The embryo transfer rate in the first cycle was 48.1% We found significant differences in the second PGS cycle if the patient had an embryo transfer in the first one ($p < 0.0001$). On the contrary, we could not find any statistical difference in the third and fourth cycles according to previous cancellations
Limitations, reasons for caution: In this study all type of patients have been included in the analysis. It would be interesting to have a bigger size sample to separate them in different groups according to the maternal age
Wider implications of the findings: There is an increase in the likelihood of having an embryo transfer in a second PGS cycle if in the previous one there were euploid embryos. The chances to have an embryo transfer for patients with more than two previous cycles are not correlated with the number of previous cancellations
Trial registration number: none

P-593 Blastocystic mosaicism: correlation analysis of chromosomal composition in different trophectoderm biopsies and inner-cell mass identifying by next-generation sequencing (NGS)

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Study question: Are euploid cells preferentially allocated near to inner-cell mass (ICM) or randomly distributed throughout the whole blastocyst? This could lead misdiagnosis in the accurate genetic screening of trophectoderm (TE) biopsy at a particular site.
Summary answer: The euploid/aneuploid cells were randomly distributed throughout the whole blastocyst, and thus the inconsistency rate between different TE biopsies to ICM were quantitatively equal.
What is known already: Trophectoderm biopsy is considered less harmful to the developing embryos, and removal of multicellular samples allows low-rate aneuploidy (a mixture of diploid and aneuploid cells between 20% to 50%) to be detected by using next-generation sequencing (NGS). However, the possibility of inconsistent ploidies between TE and ICM due to confined mosaicism (aneuploid cell lines only in a particular site) remains unclear.
Study design, size, duration: During January to December in 2015, biopsies at the six o'clock position to ICM (TE1), at the three o'clock position to ICM (TE2), and at the ICM in 33 donated blastocysts obtained from 13 randomly selected patients were analyzed on NGS platform prospectively. The inconsistency of three different biopsies in the recruited blastocysts were calculated.
Participants/materials, setting, methods: The mean age of included patients was 34.2 years, and 29 frozen-thawed blastocysts were completely diagnosed by NGS at the three different biopsy positions. The aneuploid percentage of each sample was analyzed by NGS at the finest resolution after comparing with the well-validated array comparative hybridization system: euploid (aneuploid percentage <20%), low-rate aneuploid (aneuploid percentage between 20% to 50%), aneuploid (aneuploid percentage >50%).
Main results and the role of chance: Neither ICM nor TE fractions displayed higher euploid ratio (TE1: 32.3%; TE2: 30.3%; ICM: 29.0%). Of the overall consistency in ploidies, no significant advantage was observed between any TE fractions to ICM (TE1 to ICM: 89.7% *vs.* TE2 to ICM: 90.3%); but of the aneuploid variation type, slightly increase of consistency was observed between TE2 to ICM (TE1 to ICM: 50.0% *vs.* TE2 to ICM: 60.0%). A total of 25 embryos was uniformly euploid or aneuploid in the all three biopsies (86.2%, 8 euploids and 17 aneuploids), and the mosaic blasotycts included: 1 low-rate aneuploid in the TE fractions and euploid in the ICM (3.4%); 1 euploid in the TE fractions and low-rate aneuploid in the ICM (3.4%); 1 euploid in the TE2 and low-rate aneuploid in both the TE1 and ICM (3.4%); 1 low-rate aneuploid in the TE1 and aneuploid in both the TE2 and ICM (3.4%). Therefore, the inconsistency rates of TE1 or TE2 to ICM caused by confined mosaicism were equally 10.3% (3/29).
Limitations, reasons for caution: Number of studied blastocysts can be increased.
Wider implications of the findings: The inconsistency rate of different TE biopsies to ICM identified by NGS system was obtained in this preliminary study, and it verified the randomly distribution of euploid cells in a blastocyst. The data provided the implication for further investigations of the aneuploidy and mosaicism mechanism in human embryo development.
Trial registration number: 201512096RIN, by the ethic committee of National Taiwan University Hospital

P-594 Application of next-generation sequencing technology for comprehensive aneuploidy screening of blastocysts in ART

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Study question: Can next-generation sequencing (NGS) techniques be used reliable tool for aneuploidy screening in patients undergoing IVF treatments?
Summary answer: Application of NGS in clinical preimplantation genetic screening (PGS) cycles demonstrates that this methodology is reliable to help to transfer of euploid embryos
What is known already: neuploidy is a leading cause of failure of assisted reproductive technologies programs (ART). Embryo selection with preimplantation genetic screening (PGS) improves efficiency of ART programs, increases frequency of implantation and decreases frequency of miscarriages. Currently, microarray comparative genomic hybridization (aCGH) is a commonly used method for the preimplantation screening. However, use of next-generation sequencing (NGS) is considered to be an alternative for PGS.
Study design, size, duration: Study: cross-sectional parallel evaluation, with both NGSand array-CGH techniques, of 38 blastocysts obtained from clinical PGS cycles.
Participants/materials, setting, methods: 38 patients undergoing PGS were enrolled in the study. All embryos were cultured to blastocyst stage; trophectoderm biopsy was performed on Day 5 of developmentor. The samples were analyzed using both comparative genomic hybridization and semiconductor high-throughput sequencing (Ion torrent). Whole genome amplification was carried out by PCR-based method for 28 samples and by MDA for 10 samples.
Main results and the role of chance: Comparative genomic hybridization showed that 25 samples (65.7%) had a normal karyotype, 13 samples (34.3%) had aneuploidies. In 37 samples, results of NGS were identical with aCGH, in one case the aCGH result was interpreted as 47, XXY while the NGS result was interpreted as 46, XY. This findings are due to the more strict criteria of data interpretation by aCGH in comparison with NGS. We need to elaborate the method of analysis of results of aCGH and NGS.
Limitations, reasons for caution: Further data and broad-based clinical application are required to better define the role of NGS in PGS.
Wider implications of the findings: NGS methodology may represent a valuable alternative to the other comprehensive aneuploidy screening techniques currently available. Although comparable results were obtained with both amplification methods, MDA amplification gives less noisy data. Further work is required to choose the most appropriate platform for PGS.
Trial registration number: ǰD-6043.2015.7

P-595 Accurately diagnose reciprocal translocation carrier in preimplantation human embryos by "MicroSeq" technology

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Study question: Can balanced reciprocal translocation embryos be accurately diagnosed in preimplantation genetic diagnosis (PGD)?
Summary answer: Chromosome microdissection followed by next generation sequencing can accurately identify the breakpoint and help to distinguish normal and balanced translocation embryos in PGD cycle.
What is known already: Reciprocal translocations carriers usually suffer from spontaneous abortion due to abnormal meiosis during gametogenesis. Fluorescence *in situ* hybridization (FISH)-PGD and comprehensive chromosome screening have been used to identify unbalanced embryos and prevent recurrent spontaneous abortion. However, there is no clinical applicable technique to diagnose embryos with balanced reciprocal translocations and to enable patients to choose real normal embryos preferentially transferred. Several techniques such as array based comparative genomic hybridization, next generation sequencing had been applied to characterize the breakpoints of reciprocal translocations. However, to our knowledge no clinical trials have been performed to diagnose balanced reciprocal translocation embryos in PGD.

Study design, size, duration: This clinical diagnostic study included totally 8 couples with balanced reciprocal translocation in CITIC-XIANGYA from July 1, 2014 to December 31, 2015. Breakpoints and surrounding SNP haplotypes were characterized before oocyte retrieval. Results of balanced reciprocal translocation embryos were compared to prenatal diagnosis results to determine the accuracy.

Participants/materials, setting, methods: Eight patients with balanced translocation participated in the study after signing informed consent. We combined chromosome microdissection and NGS (MicroSeq) to identify the translocation breakpoints and their surrounding SNP haplotype. With the information of the breakpoint and SNP haplotype, specific primers were designed and used for analyzing DNA amplified samples of 37 blastocysts from the 8 couples. The PCR results were then compared with the PGD-CCS and prenatal diagnosis results of the analyzed blastocysts.

Main results and the role of chance: We characterized balanced translocated chromosomes in 8 patients by MicroSeq. The precise breakpoint could not be characterized in only one patient because the breakpoint is located in 22q11.21, which harbored a complicated palindrome structure. However, haplotype surrounding the breakpoints in 8 patients were successfully characterized. Interestingly, translocations in 6/7 patients with fully characterized balanced translocations cause gene disruption. We then analyzed 37 blastomeres by both PGD-CCS and breakpoint-specific PCR with the breakpoint and haplotype information. Among them, 26 blastomeres were characterized aneuploidy by PGD-CCS. As predicted by PGD-CCS, 2/26 embryos should have two translocated chromosomes, 23/26 embryos should have one translocated chromosome, and 1/26 should not have any translocated chromosome. Our breakpoint analysis result was consistent with PGD-CCS prediction. Furthermore, we characterized the 11/37 "normal" embryos diagnosed by breakpoint-specific PCR. 9/11 embryos had two breakpoints, which implicated they were embryos with balanced translocations (balanced embryos). No breakpoint could be detected in another 2 embryos, implicated that they were real normal embryos. 4/9 balanced embryos and 2/2 normal embryos were transferred. 4 patients got pregnant. By prenatal diagnosis, 3 babies were confirmed balanced translocation carriers and one was normal without balanced translocation, which consisted with our breakpoint analysis.

Limitations, reasons for caution: One limitation of the study is failure to confirm the PGD results in some embryos due to implantation failure and ethical reasons. MicroSeq could not accurately find the breakpoints in regions which harbored complicated palindrome structures. However, SNP haplotype around the breakpoint region help to diagnose balanced translocation embryos.

Wider implications of the findings: This study showed that the genetic influence of balanced translocations might be underestimated since a significant portion (85.7%) of chromosome translocations caused gene disruption in this study. Characterizing surrounding SNP haplotypes of the breakpoints by MicroSeq could help to diagnose embryos with Robosonian's balanced translocation in the future.

Trial registration number: NA.

P-596 Reduced mRNA levels and isoforms of the FMR1 gene in infertile women with low CGGnn <26 repeats

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Study question: Do FMR1 mRNA levels and isoforms differ in infertile women between *low FMR1* $CGG_{n<26}$ and *non-low* $CGG_{n\geq26}$ repeats?

Summary answer: Containing a mixture of isoforms, *FMR1* message RNA levels, are significantly reduced in women with *low FMR1* $_{n<26}$ compared to those with *non-low FMR1*$_{n\geq26}$ repeats.

What is known already: The fragile X mental retardation 1 (*FMR1*) gene exists in various mutations, defined by CGG_n, which have been reported to be associated with distinct ovarian aging patterns and varying IVF outcomes. Most profoundly associated with early ovarian aging and negative reproductive outcomes appears to be the *low* $CGG_{n<26}$ mutation. Moreover, distinct alternative splicing and various FMR1 mRNAs/proteins of the gene have been reported.

Study design, size, duration: We prospectively studied luteinized granulosa cells obtained in IVF cycles during 2014/2015, and investigated whether amounts of *FMR1* mRNA and its isoforms differ in presence of different *FMR1* mutations. Mural granulosa cells were collected on day of retrieval.

Participants/materials, setting, methods: 62 IVF patients were studied. CGG_n is at our center routinely determined at initial presentation, utilizing a commercial assay. Definition as *low* mutation ($n = 25$) required at least one $CGG_{n<26}$ allele, while a *non-low* patient ($n = 37$) was defined by both alleles being $CGG_{n\geq26}$. Message RNAs were extracted and reverse transcribed to cDNA. Various FMR1 isoforms were examined by quantitative PCR using 5 sets of primers targeting middle and C-terminal regions of FMRP protein sequences.

Main results and the role of chance: All here examined 5 groups of FMR1 isoforms were found significantly differently expressed in mural granulosa cells of *low* and *non-low* groups, with all isoforms in the *low FMR1* group found significantly reduced ($P = 0.011$, $= 0.045$, $= 0.003$, $= 0.009$, $= 0.005$, respectively).

Limitations, reasons for caution: The sample size of this still ongoing study was too small to assess individual mutations within the *non-low* study group, such as *normal* (both alleles within $CGG_{n = 26–34}$) and homozygous high/high (both alleles at $CGG_{n>34}$).

Wider implications of the findings: Gene expression has been reported at all follicle maturation stages in rat. Difference in mRNA levels and isoform distribution of the *FMR1* gene may regulate the translation and cellular localization within follicles of FMRP, leading to dysfunction of FMRP proteins, affecting ovarian aging and outcomes differently with different *FMR1* mutations.

Trial registration number: N/A

P-597 Preimplantation genetic diagnosis (PGD) for translocation carriers using whole genome screening by microarray analysis at the blastocyst stage

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Study question: What is the value of whole genome screening by array comparative genomic hybridization (CGH) for PGD in embryos of translocation carriers?

Summary answer: Array CGH for PGD of translocation carriers is a beneficial clinical application to identify viable euploid embryos for transfer.

What is known already: Translocation carriers usually have a normal phenotype, but through the generation of unbalanced gametes from impaired chromosome segregation they have an increased risk of having fertility problems, recurrent miscarriages or an abnormal chromosomal offspring. Recent embryological and genetic technical advances in the field of PGD have improved the means of selecting a balanced or unaffected embryo, suggesting a benefit to the pregnancy rates for couples with chromosome aberrations.

Study design, size, duration: This is a retrospective data analysis study contacted from October 2013 to December 2015. A total of 224 blastocysts originating from 50 PGD oocyte retrieval cycles were investigated. These cycles involved 34 couples with a mean maternal age of 32.5 years with one of both parents being a carrier of Robertsonian translocations ($n = 9$), of reciprocal translocations ($n = 21$), of inversions ($n = 2$), of an insertional translocation ($n = 1$) and of a double two-way exchange reciprocal translocation ($n = 1$).

Participants/materials, setting, methods: Trophectoderm biopsy and vitrification of embryos was performed on the 5th or 6th day of development. Whole genome amplification (WGA) was performed on all samples, and the amplified DNA from 207 blastocysts was analyzed with array CGH via the 24sure+microarrays (Bluegnome, Illumina). Embryos with normal chromosome content were warmed and transferred to the patients in natural, non-stimulated cycles.

Main results and the role of chance: We detected chromosome abnormalities in 133/207 embryos (64.2% of successfully amplified) while 74 showed

a normal microarray profile (35.7%). In 48 of the 133 abnormal embryos (36.1%), an unbalanced rearrangement originating from the parental translocation was identified. Interestingly, 34.6% of the abnormal embryos (46/133) harbored *de novo* chromosome aberrations that were not directly linked to the parental translocation in question. We also detected a combination of unbalanced – parental derived – rearrangements and *de novo* chromosome aberrations in 27/133 abnormal embryos (20.3%). More specifically, from a total of 223 chromosome abnormalities detected, we identified 78 aneuploidies and 23 structural abnormalities (a combined 45.3%), unrelated to the parental rearrangement. Due to technical limitations, results were not generated in 12/207 (5.8%) of the samples. The pregnancy rate per embryo transfer, corresponding to the detection of hCG at least 15 days after embryo replacement, was 43.9%. Our preliminary data are in favor of the implementation of comprehensive chromosome screening (CCS) on trophectoderm biopsies for PGD, enabling better embryo selection and possibly leading to a higher pregnancy rate.
Limitations, reasons for caution: Besides the detection of chromosomal aberrations due to the parental translocation, this approach revealed a high occurrence of aneuploidies and structural rearrangements unrelated to the parental translocation. It is a retrospective data analysis study with a small cohort size.
Wider implications of the findings: Use of array CGH for PGD on trophectoderm biopsy at the blastocyst stage is more convenient than fluorescent *in situ* hybridization (FISH) and leads to a more reliable estimate of the genomic content of the embryo compared to single or double cell biopsy at the cleavage stage.
Trial registration number: n/a

P-598 Common variants near FSHB and in SMAD3 provide insight into human dizygotic twinning and female fertility and reproduction

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Study question: Can we identify the genetic basis of spontaneous dizygotic (DZ) twinning by establishing a Twinning consortium for genetic association studies?
Summary answer: We identified the first robust genetic variants for DZ twinning. These variants are significantly associated with a broad range of fertility traits in women.
What is known already: The tendency to conceive spontaneous DZ twins is a complex trait that aggregates in families, and there is evidence that it is at least, in part, genetically determined. Although the fundamental physiological mechanism for DZ twinning is the release and fertilization of multiple oocytes, for which both animal models and human data suggest multifactorial inheritance, the underlying molecular basis of the trait is not understood. So far, efforts to find genetic factors that contribute to DZ twinning in humans have not been successful.
Study design, size, duration: We performed the first genome-wide association study (GWAS) for being a mother of DZ twins in 1,980 mothers of spontaneous DZ twins and 12,953 controls from The Netherlands, US and Australian European ancestry cohorts. 9,073,348 SNPs (after imputation against the 1000G reference set) were tested. Findings were replicated in a large Icelandic cohort (3,597 mothers of twins and 297,348 controls) and tested for association across a broad range of fertility traits in women.
Participants/materials, setting, methods: All cases underwent screening to exclude mothers who received assisted reproductive techniques. Controls were screened to exclude pedigrees containing DZ twins. The mean maternal age at delivery was 29.3 (SD = 4.5) for the discovery cohorts and 30.3 (SD = 4.5) for the replication cohort.
Main results and the role of chance: Two single nucleotide polymorphisms (SNPs) were identified (rs11031006 near FSHB, $p = 1.54 \times 10^{-9}$, and rs17293443 in SMAD3, $p = 1.57 \times 10^{-8}$) and replicated ($p = 3 \times 10^{-3}$ and $p = 1.44 \times 10^{-4}$, respectively). Based on ~90,000 births in Iceland, the relative risk of a mother delivering twins increased by 18% for each copy of the rs11031006-G allele, and 9% for the rs17293443-C allele. A higher polygenic risk score (PRS, or cumulative genetic risk profiles from across the genome) for DZ twinning, calculated based on the results of the DZ twinning GWAS, was significantly associated with DZ twinning in Iceland ($p = 0.001$). A higher PRS was also associated with having children ($p = 0.01$), greater lifetime parity ($p = 0.03$) and earlier age at first child ($p = 0.02$). The rs11031006-G allele was associated with higher serum follicle-stimulating hormone (FSH) levels, earlier age at menarche, earlier age at first child, higher lifetime parity, lower polycystic ovary syndrome risk, and earlier age at menopause. Conversely, the rs17293443-C allele was associated with later age at last child.
Limitations, reasons for caution: NA.
Wider implications of the findings: This study extends our understanding of the control of natural multiple follicle growth and reproductive aging. This new knowledge will contribute to the development of novel ways to manipulate ovarian functioning, which is crucial for the treatment of infertility as well as for contraception.
Trial registration number: na

P-599 A comparison of transcriptomic profiles in endometrium during window of implantation between women with unexplained recurrent implantation failure and recurrent miscarriage

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Study question: Are there any differences in the endometrial transcriptome profiles in the window of implantation among women with unexplained recurrent implantation failure, recurrent miscarriage and normal fertile control?
Summary answer: There were significant differences in transcriptomic profiles between women with unexplained recurrent implantation failure (RIF) and recurrent miscarriage (RM).
What is known already: The endometrium becomes receptive to the embryo only in the mid-luteal phase of the menstrual cycle. Endometrial factors play an important role in implantation. Extensive studies have shown that women with recurrent miscarriage or recurrent implantation failure have altered expression of individual receptivity markers during the window of implantation.
Study design, size, duration: There were a total of 24 subjects recruited, 9 women with unexplained recurrent implantation failures (RIF), 11 women with recurrent miscarriage (RM) and 4 fertile subjects.
Participants/materials, setting, methods: Endometrium samples were collected exactly 7 days after luteal hormone surge (LH+7). The transcriptome was determined by RNA-Seq using 14 samples (5 RIF, 6 RM, and 3 controls). Differential gene expression validation was confirmed by quantitative PCR using all 24 samples.
Main results and the role of chance: Expression profiles of RIF and RM can be separated by principle component analysis. Genes contributed to the biological difference between RIF and RM was identified. Among all molecular pathways C3, CD55, C4A, C4BPA, CFD, and SERPING1 in complement cascade were highly expressed in RIF samples; while cell signaling seemed to be significantly activated in RM patients during WOI. The significant differences between RIF and RM suggested that the underlying mechanisms of unexplained recurrent implantation failure are different from unexplained recurrent miscarriage.
Limitations, reasons for caution: More functional studies are needed to validate the role of complement pathway in implantation.

Wider implications of the findings: RIF and RM may be the result of defective implantation at different stages of early pregnancy. Identification of the pathways involved in each process would enable the differential design of treatments for each patient group.
Trial registration number: None.

P-600 Influence of genetic polymorphisms associated with ovarian response on clinical outcomes within an oocyte donation program

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Study question: Does genetic polymorphisms affect ovarian response and clinical results in an oocyte donor program?
Summary answer: There is currently no evidence that genetic polymorphisms are associated with ovarian response to controlled ovarian stimulation in a population of fertile egg donors,
What is known already: Ovarian response to gonadotropins is characterized by a wider inter-individual variability, with low responders with few or no mature follicle, and high responder characterized by an increased risk of developing a syndrome of ovarian hyperstimulation.

In assisted reproductive treatments, the stimulation protocol for low/high ovarian response to exogenous FSH is individualized, although the response to intense stimulation with gonadotropins is difficult to predict. However, it has been suggested that one of the reasons for this variability of ovarian response to stimulation protocols lies in the genetic status of the patient
Study design, size, duration: 70 oocyte donors were subjected to ovarian stimulation protocol. After oocyte recovery, they were classified into three groups depending on the number of oocytes obtained: low response (≤6 oocytes), normal-response (7–15 oocytes) and high response (>15 oocytes). Then we identified genetic polymorphisms associated with FSH receptor gene, the β-LH subunit, CYP19A1, estrogen receptor, GDF-9 and BMP-15 for the purpose of describing a suitable genetic profile that helps predict most effective way ovarian response
Participants/materials, setting, methods: Subjects were assigned to receive daily doses of 150 UI rFSH; from day 6 of stimulation onwards, 0.25 mg GnRH antagonist were administered and a single dose of GnRH agonist (0.2 mg) was administered for triggering final oocyte maturation. Oocyte pick-up was programmed 36 h after inducing ovulation. Oral epithelial cells were taken using FTA cards; the further analysis of genetic polymorphisms was performed by PCR with Taqman probes on a device StepOne Plus
Main results and the role of chance: The distribution was 15.7% for low-responders, 37.1% for normal-responders and 47.1% for high-responders. The age was 23 to 33 (28.09 ± 3.08 years), 18 to 35 (26.54 ± 4.95 years), 18 to 32 years (24.52 ± 3.73 years) and the estradiol levels were 881.78 ± 1530.12 pg/ml, 2343.04 ± 1389.45 pg/ml 3249.06 ± 1538.95 pg/ml, respectively. The age in the low-response group was significantly older than in the high-responder group ($p = 0.031$). The estradiol levels were different in the low-response group vs. normal and high-responders ($p = 0.035$ and $p < 0.001$; respectively).

All the genotype frequencies were in the Hardy-Weinberg equilibrium.

Univariate analysis revealed that any polymorphism was associated with ovarian response: FSHR T307A (rs6165) ($p = 0.359$); FSHR N680S (rs6166) ($p = 0.343$); LHB W8R (rs1800447) ($p = 0.160$); ESR1 Pvu T/C (rs2234963) ($p = 0.234$); ESR1 Xba A/G (rs9340799) ($p = 0.369$); LHB I15T (rs34349826) ($p = 0.160$); CYP19A1 (TTTA)*n* (rs57921193) ($p = 0.094$); CYP19A1 (I/D) (rs11575899) ($p = 0.770$), GDF9 546G/A (rs10491279) ($p = 0.630$); BMP15 −9C/G (rs3810682) ($p = 0.566$). On the other hand, we have shown an association statistically significant for LHB with the number of follicles ($p = 0.036$; for both LHB polymorphisms). There was not significance with the number of oocytes.
Limitations, reasons for caution: This study has been performed in oocyte donors, which form a fairly homogeneous group in terms of age and ovarian response, so these results may not be extrapolated to other groups of women undergoing an assisted reproductive treatment.
Wider implications of the findings: Although data are accumulating with evidence suggesting that the ovarian response to controlled ovarian stimulation is mediated by genetic polymorphisms, these biomarkers are not determining factors in predicting ovarian response to the administration of exogenous gonadotropins when stimulating oocyte donors. However, more data are needed to draw firm conclusions
Trial registration number: None.

P-601 Chromosome abnormalities detected by array comparative genomic hybridization and next-generation sequencing: results on >38,000 embryos

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Study question: Are there significantly different chromosome abnormality rates, controlled by age, between PGS next-generation sequencing (NGS) using the VeriSeq assay and array comparative genomic hybridization (aCGH)?
Summary answer: NGS is a more accurate method of identifying euploid embryos across all age groups due to its ability to detect mosaicism in trophectoderm biopsies.
What is known already: SART success rates can be enhanced by implementing PGS as shown by recent randomized trials. The newest technique for PGS is next generation sequencing (NGS), which has the potential to provide additional information about the embryo beyond chromosomal aneuploidy (specifically polyploidy and mosaicism) and may offer higher accuracy for aneuploidy detection. NGS techniques should be valued over the current PGS gold-standard of array-CGH and offer increased precision and higher analytical depth to substitute aCGH.
Study design, size, duration: Sureplex whole genome amplification (WGA) products of 38,909 blastocyst biopsies were assessed. 24,507 biopsied samples comprising 4,981 cases for aCGH testing were received from 161 fertility centers between 01/01/2015 and 12/11/2015. 14,702 biopsied samples comprising 2,861 cases for NGS testing were received from 73 fertility centers between 01/28/2014 and 12/11/2015.
Participants/materials, setting, methods: SurePlex WGA products of biopsies were processed using Illumina 24 sure microarrays for aCGH or VeriSeq PGS assay with the MiSeq sequencing platform for NGS. Both results were interpreted through BlueFuse Multi analysis software and diagnosed as euploid or abnormal for aCGH or euploid, abnormal, or mosaic for NGS, and distributed in age groups following guidelines set by Society for Assisted Reproductive Technology (SART).
Main results and the role of chance: Results are shown in the below table. NGS yielded a lower proportion of euploid embryos available for transfer across all age groups tested. The ability of NGS testing to identify mosaicism, which aCGH is less able to do, accounts for a large proportion of this discrepancy.

Age Group	[a]CGH-# Euploid Embryos	[a]CGH-Total analysed	[a]CGH-% Euploid	NGS-# Euploid Embryos	NGS-Total Analysed	NGS-% Euploid	NGS-% Mosaic
Egg donors	2863	4416	64.83%	2095	3347	62.59%	11.35%
< 35	4461	7421	60.11%	1904	4091	46.54%	22.66%
35–37	2410	4561	52.84%	1081	2682	40.31%	19.02%
38–40	1751	4538	38.59%	808	2683	30.12%	13.38%
41–42	521	2252	23.13%	210	1239	16.95%	10.57%
>42	142	1019	13.94%	71	660	10.76%	9.70%

Limitations, reasons for caution: The results are an assessment of abnormalities detected in different age groups within a large study. It is not a direct comparison of the same embryos analysed by both methods which has already been published.
Wider implications of the findings: Mosaic blastocysts have lower potential of implantation, higher risk of miscarriage and more likely to result in babies with congenital abnormalities. NGS distinguishes better between mosaic and euploid embryos, thereby increasing the selection of embryos with higher implantation potential, avoiding misclassifying mosaics as euploid (false negative) or aneuploid (false positive).
Trial registration number: N/A.

P-602 Genetic screening analysis in infertility patients: a retrospective single center review

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Study question: The objective of this study was to examine the merit of pan-ethnic genetic screening of infertility patients at a single fertility center.
Summary answer: Wide genetic screening of infertility patients is justified by the frequent discovery of carrier status, and the small yet significant percentage of dual carrier state.
What is known already: Of over 25,000 genes identified, the average human is a carrier for 5–10 single gene mutations. Several panels have been developed to include a wider range of genes in order to further reduce the chance of having a child affected with even the rarest genetic conditions. Recent improvement in PGD (Pre-implantation genetic diagnosis), have led to greater precision in diagnosis of single gene mutations. This approach, of wide screening of patients who are candidates for fertility treatment, especially IVF, followed by exclusion of affected embryos through PGD, holds great promise in reducing the genetic load of the next generation.
Study design, size, duration: A retrospective cohort study of 4279 infertility patients who underwent the same genetic screening at our fertility center between June 2013 and July 2015.
Participants/materials, setting, methods: Serum samples from each patient were tested for a pan-ethnic panel of 102 genes (Counsyl®, San Francisco). This panel was selected based on incidence in the population as well as clinical significance.
Main results and the role of chance: 29% (1252/4279) of patients were found to be carriers of at least one autosomal recessive genetic mutation. The five most common mutations included: Hb beta chain-related Hemoglobinopathy (135/4279, 3.2%), Cystic Fibrosis (134/4279, 3.1%), Pseudocholinesterase Deficiency (103/4279, 2.4%), Spinal Muscular Atrophy (85/4279, 1.9%) and GJB2-related DFNB1 nonsyndromic hearing loss and deafness (74/4279, 1.7%). Out of all patients, 13% (585/4279) were found to be carriers of mutations that may cause physical symptoms. In 1.6% (15/948) of the couples, both male and female partners carried mutations for the same gene. All of the latter underwent pre-implantation genetic diagnosis (PGD).
Limitations, reasons for caution: Not applicable.
Wider implications of the findings: Genetic screening in fertility patients helps prevent the unexpected occurrence of a genetic disease in the offspring. The pan-ethnic panel abandons patients' often inaccurate reporting of their genetic heritage. In <2% of cases, both partners carried the same gene and used PGD to prevent their children from being affected.
Trial registration number: NA.

P-603 Short sperm telomere length is associated with patients who are overweight or obese: A possible mechanism for poor IVF treatment outcomes

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Study question: Whether sperm telomere length (STL) is related to the obesity? The impacts of men's body mass index (BMI) on STL, early embryo quality, clinical outcomes in couples undergoing IVF.
Summary answer: Short sperm telomere length is associated with patients who are overweight or obese.
What is known already: Our previous study showed that STL is positively associated early embryonic development. And other studies found that leukocyte telomere length (LTL) is shorter in obesity compared with normal weight people.
Study design, size, duration: 651 couples were recruited from August 2013 to August 2015, including 345 males with normal weight and 306 males with abnormal weight (normal group: 20–25 kg/m^2; abnormal group: >28 kg/m^2).
Participants/materials, setting, methods: Fertilization rate, embryo quality and pregnancy rate were compared between two groups. The average STL was determined in the genomic DNA by using a real-time PCR for each patient. A standardized protocol was used to perform a photometric nitro blue tetrazolium (NBT) assay to measure seminal ROS production. A sperm chromatin dispersion (SCD) kit was used to detect DNA fragmentation rate in the sperm according to the manufacturer's recommendations.
Main results and the role of chance: Couples with male's BMI over 28 kg/m^2 exhibited significant lower fertilization rate ($p = 0.002$), good-quality embryo rate ($p = 0.044$) and clinical pregnancy rate ($p = 0.038$) compared to their normal weight counterparts. In a multivariable logistic regression analysis adjusting for female age, female BMI, basic FSH level, male age, number of embryos transferred and sperm count, male BMI over 28 kg/m^2 was associated with lower clinical pregnancy rate (odds ratio 0.75 [95% CI: 0.61–0.88]; $p < 0.01$). The mean STL in the abnormal BMI group was also significantly shorter than that of normal weight group ($p < 0.001$). In addition, individuals with abnormal BMI had higher ROS (Reactive oxygen species) content ($p < 0.01$) and sperm DNA fragmentation rate ($p < 0.01$) when compared with normal BMI. And the mitochondrial activity was also lower in the normal BMI group than that of abnormal BMI group.
Limitations, reasons for caution: This study showed that short sperm telomere length is associated with patients who are overweight or obese. Additional studies are needed to confirm these observations.
Wider implications of the findings: STL has the potential to be used as a marker for the prediction of embryonic quality for the patients who are overweight or obese.
Trial registration number: none.

P-604 Polygenic profiles for predicting early menopause

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Study question: Can we use polygenic risk scores to predict the risk for early menopause, before age 45?
Summary answer: Genetic risk profiles including approximately 20,000 markers have a good predictive value for assessing the risk of early menopause.
What is known already: Reproductive aging has an impact of female fertility and general health, and involves a considerable genetic component as evidenced by recent genome-wide association studies (GWAS). Currently, no genetic markers are used for predicting menopausal age.
Study design, size, duration: Retrospective cohort study.
Participants/materials, setting, methods: Polygenic risk scores were generated using the publicly available ReproGen consortium menopausal age GWAS meta-analysis summary statistics including data for 2.4 million markers and involving approximately 70,000 women. Correlation between risk scores and menopausal age was tested among 3,189 post-menopausal women (at least 45-year-old) in the Estonian Biobank. Receiver operating characteristic (ROC) curves were generated to evaluate the predictive value of genetic risk scores for discriminating women with early menopause.
Main results and the role of chance: A genetic risk profile including 19,683 markers was significantly correlated with age at natural menopause ($r^2 = 0.4$, $p = 4 \times 10^{-82}$). The same profile was a good predictor of early menopause (AUC = 0.70), outperforming the predictive value of smoking status (AUC = 0.55), which is one of the most important lifestyle factors affecting menopausal age. Combining genetic risk scores and smoking status improved model performance only slightly. Women among the top 10% of the genetic risk score had a relative risk of 2.80 for early menopause (the risk for early menopause of 14.0%), compared to the population average (5%), while women with the lowest 10% of scores had a relative risk of 0.30 (the risk for early menopause of 1.5%). When the extremes of the genetic risk score were compared, more than a 9-fold difference (14.0 vs 1.5%) in risk for early menopause was observed.
Limitations, reasons for caution: Genetic risk profiles need further testing in combination with hormonal and ultrasound markers currently used for assessing ovarian reserve and predicting menopause.
Wider implications of the findings: Polygenic profiles show considerable discriminative power for detecting women at risk of early menopause and age-related infertility, and therefore have the potential to increase the accuracy of ovarian reserve assessment, leading to more personalized counselling regarding family planning and patient management (like the use of assisted reproduction and oocyte cryobanking).
Trial registration number: NA.

P-605 Progesterone receptor gene is involved in recurrent implantation failure

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Study question: Could any polymorphic variant of human progesterone receptor (PGR) gene be used as molecular biomarker in recurrent implantation failure (RIF)?
Summary answer: Next-generation sequencing (NGS) of PGR gene has identified two polymorphic variants that could be involved in implantation failure.
What is known already: It is known that PGR mediates the action of progesterone hormone and both are critical for development and maintenance of the endometrium and consequently for implantation of embryos after in vitro fertilization (IVF). Since RIF is the most frequent cause of lack of pregnancy after IVF and some polymorphic variants of PGR have been implicated in this context, it is important an enhanced understanding of these variants and their roles within the uterine compartments.
Study design, size, duration: A cross-sectional study was conducted with blood samples of 48 women presenting recurrent implantation failure (RIF) subjected to IVF/ICSI protocols and 46 fertile women (control group). RIF group - inclusion criteria: ≥2 failed IVF/ICSI attempts/≥5 embryos transferred, age ≤ 39 years and normal karyotype; exclusion criteria: uterine defects, ultrasonographic evidence of hydrosalpinx, infections, endocrine problems, coagulation defects or thrombophilia and autoimmune defects. Control group - volunteers with at least 1 live birth, without treatment and no history of miscarriage.
Participants/materials, setting, methods: Genomic DNA was extracted from the peripheral blood of RIF and control groups. The coding regions (exons) and no translated variants in both 3'/5'UTRs of PGR gene were analyzed by NGS using TruSeq Custom Amplicon/MiSeq-Systems. Raw paired-end reads were aligned to human reference genome GRCh37 using BWAv0.7.12. Reads were re-aligned around indels and quality scores re-calibrated using GATK–(v3.5-0-g36282e4). GATK's UnifiedGenotyper was used as a variant caller to detect Single Nucleotide Variations (SNVs) on the full alignments.
Main results and the role of chance: Following the best practices of GATK for amplicon sequencing we found two prevalent variants in RIF group and their frequencies are shown in Table 1. The genotypic frequencies of these variants in women presenting RIF and in controls subject are shown in Table 2. A significant difference between RIF and control group ($p = 0.03$) was found by chi-square test.

Table 1. Variants frequencies

Gene	rs	allele	Control	Control	RIF	RIF
			n	Minor allele frequency	*n*	Minor allele frequency
PGR	rs1042838	C>A	8	0.092	15	0.173
PGR	rs11224563	C>T	8	0.094	15	0.176

Table 2. Genotypic frequencies (PGR gene)

	*RIF [*n*(%)]	Control [*n*(%)]	*p*-value
rs1042838			
C/C	32 (68.1)	38 (82.6)	0.03
C/A	15 (31.9)	6 (13.0)	
A/A	0 (0.0)	2 (4.4)	
rs11224563			
C/C	32 (68.1)	38 (82.6)	0.03
C/T	15 (31.9)	6 (13.0)	
T/T	0 (0.0)	2 (4.4)	

*01 sample in RIF group was not mapped.

Limitations, reasons for caution: More studies are necessary to confirm this data considering variations in different ethnic population.
Wider implications of the findings: Taking into account that PGR variants (rs1042838 and rs11224563) may be involved with recurrent implantation failure, the expansion of this research should be considered.
Trial registration number: Not applicable. The local ethics committee authorized this study.

P-606 Age effect on male gamete performance through apoptotic and DNA repair gene expression

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Study question: We question the effect of age on the sperm's ability to generate offspring by assessing DNA repair and apoptosis regulating genes.
Summary answer: Transcriptome profiling utilizing RNA-Seq in human spermatozoa reveals that age sensitive genes related to DNA repair and apoptosis is indicative of reproductive outcome.
What is known already: It has been known gametes produced by an aging male may suffer increased aneuploidy and compromised chromatin integrity. Standard semen parameters, although useful in the diagnosis, are not predictive of male fertility and are not very useful in cases of idiopathic infertility. Upon fertilization, the sperm provides a complete, highly structured, and epigenetically marked genome that plays a distinct role in embryonic development. Characterization of sperm RNAs by next generation sequencing through gene expression may be suited to better understand the effect of age on the ability of the male gamete to generate offspring.
Study design, size, duration: In a 12-month period, we assessed the effect of age on male gamete integrity and its ability to participate in embryo development. The analysis was carried out by measuring the expression of DNA repair ($n = 4$) and apoptotic modulating ($n = 3$) genes. RNA extraction from 26 semen specimens was carried out on men undergoing infertility screening, with 19 men in the study group and compared to 7 men acting as a control.
Participants/materials, setting, methods: An average of 25×10^6 human spermatozoa was used to isolate total RNA using a spin column commercial kit. The nucleic acid quality and spermatozoal RNA concentration was assessed. The RNA samples were then made into paired-end libraries. Pilot paired-end 76bp RNA-Sequencing (RNA-Seq) using an Illumina platform (NextSeq 500) was carried out and expanded to 60M reads. Expression values are calculated in Fragments Per Kilobase Of Exon Per Million Fragments Mapped (FPKM) and normalized read counts.
Main results and the role of chance: A total of 7 men with an average age of 26 ± 5 years presented with a sperm concentration of 27.3 ± 27, and motility of 46.6 ± 24, and morphology of 3.0 ± 2. Sperm RNA-Seq, and expressed as FPKM, was carried out and after differential analysis patients were plotted in function of male age. We found that a DNA repair gene cluster *(APLF*, *CYB5R4*, *ERCC4* and *TNRFSF21)* and an apoptotic modulating gene cluster (*MORC1*, *PIWIL1* and *ZFAND6*) had a higher expression in younger patients (47.2 ± 15 FPKM) that progressively declined with advancing male age (9.5 ± 13 FPKM) ($p = 0.02$). This evidenced a clear inverse correlation between gene expression (FPKM) and male age. Once the reproductive potential as the ability to achieve a pregnancy was assessed, it clearly evidenced that the loss of expression of these genes was accompanied by the inability to procreate. A conserved gene expression for the specific genes analyzed confirmed the reproductive capacity and indicated a threshold at 30 years of age.
Limitations, reasons for caution: This study is part of an ongoing investigation to profile men undergoing male infertility screening and must be confirmed in a larger cohort. Gene expression profiling of infertile men may serve as an assay to measure the reproductive potential of the male gamete.
Wider implications of the findings: Sperm RNA-Seq is a reliable and reproducible technique that may aid in the diagnosis of men undergoing infertility screening. DNA repair and apoptotic genes play a key role in body development and function. This notion may clarify the cause of infertility that cannot be predicted solely by standard semen analysis.
Trial registration number: N/A.

P-607 Practical benefits of re-examination of blastocysts using next-generation sequencing technology (NGS)

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Study question: To evaluate the clinical value of re-examination of the test-failure blastocysts in preimplantation genetic diagnosis cycles.
Summary answer: Test-failure blastocysts that survived after warming, can be rebiopsied, vitrified and warmed, and have a high chance to be euploid and lead to live births.
What is known already: The test-failure rate of embryos in PGD/PGS cycles depends on diagnostic method and is approximately 5–8%. The low concentration of WGA product, dead trophectoderm (TE) cells from morphologically poor embryos, damage of nuclei after biopsy may affect the quality of results. Blastocysts after biopsy need to be frozen and transferred in the frozen cycle. The most reports are about biopsy and freezing the embryos at two different stage: cleavage-stage and blastocyst. The tolerance of blastocysts to undergo the second round of biopsy, vitrification and warming and their implantation potential has not been fully investigated.
Study design, size, duration: Retrospective study including the data analysis of 299 PGD/PGS cycles involving blastocysts stage embryos performed between November 2014 and September 2015 at INVICTA Fertility Centre, Poland. During that period a total of 1224 blastocysts were evaluated with the NGS protocol. Of those, 75 blastocysts in 52 cycles showed test-failure (6.5%).
Participants/materials, setting, methods: 75 test-failure blastocysts were re-examined. Of the 75 warmed embryos, 43 completely expanded after 2–6 h and 11 overnight. 21 blastocyst (28%) did not expand. All 54 expanded blastocysts were successfully rebiopsied and retested using NGS technology. The method involved whole genome amplification (WGA) before NGS protocol. Ion Torrent Suite Software and Invicta Bioinformatics Team Script were used for chromosome copy number variation analysis.
Main results and the role of chance: All 75 blastocysts were graded based on the trophectoderm quality (scored with TE grades A–C) before the first biopsy. The expansion rates after warming in TE grade A, B and C groups were 94.4%, 70% and 59.3% respectively. Among the 54 rebiopsied blastocysts, in the case of 9 (16.7%) embryos there was no product after WGA. 19 blastocysts had normal chromosomal status (35.2%), 26 blastocysts were abnormal (48.1%). The rates of normal chromosomal status in blastocysts with TE grade A, B, and C were 43.8%, 56.3% and 23.1% respectively and they did not differ significantly among the three groups. Normal blastocysts were transferred in 13 frozen single embryo transfer cycles. Of the 19 re-examined euploid blastocysts 17 survived (89.5%). Implantation rate in TE grade A, B and C was 57.1%, 25% and 0% respectively. Single clinical pregnancies were found in 6 cases (46.2%). Four healthy infants were born and one pregnancy is still ongoing. One miscarriage was noted at 11 weeks.
Limitations, reasons for caution: The study is limited by sample size. A higher sample size or a prospective randomized design could be used in future studies to corroborate the current findings.
Wider implications of the findings: The test-failure blastocysts still have a high percentage of normal chromosomal status. Blastocysts can survive two rounds of biopsies, vitrification and warming and may have a good implantation potential. Re-examining blastocysts that have failed the initial test, particularly those with high TE quality, may be clinically valuable in PGD/PGS cycles.
Trial registration number: not applicable.

P-608 Gene expression of apoptotic markers in cumulus and granulose cells is altered in young women with reduced ovarian reserve

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Study question: Is gene expression of apoptotic markers altered in cumulus (CC) and granulose cells (GC) from young women with reduced ovarian reserve compared with oocyte donors?
Summary answer: An imbalance of apoptosis in favor of proapoptotic markers was observed in CCs and GCs from low ovarian reserve young women compared with oocyte donors.
What is known already: CCs and GCs transcriptome has been studied to identify multiple genes that are differentially expressed in CC and GCs surrounding a good- versus a poor quality oocyte and several microarray studies have been performed in order to identify biomarkers associated with oocyte quality. However, to date, there is no consensus as to which genes are the most useful as biomarkers of oocyte competence and limited knowledge exists about changes in apoptotic markers in GC and CC associated with low ovarian reserve (LOR).
Study design, size, duration: This observational, prospective study compared the mRNA expression of 92 apoptotic markers in 185 oocyte-cumulus and 114 oocyte-granulose complexes retrieved from 3 healthy fertile oocyte donors <35 years old and 3 patients <35 years old who had a low response (≤5 oocytes retrieved) after gonadotropic stimulation from June to November 2015. Only donors with at least a previous cycle with pregnancy were included.
Participants/materials, setting, methods: All patients were stimulated with the same protocol (FSHr and triggering with GnRH analogues). GCs and CCs were collected from each patient and immediately fresh-frozen. mRNA was extracted using commercial kits and the mRNA expression of 92 apoptotic genes and 4 endogenous controls was measured by qRT-PCR using microarray plates. No parametric tests were used in order to identify any significant difference between the groups. Statistical significance was set at $p < 0.05$.
Main results and the role of chance: Sixteen mRNA were significantly different between donors and patients. NAIP, TBK1, BAD, BAX, CASP2, CASP3, CASP4 and CASP10 were significantly elevated in patients compared with donors. By the contrary, FAD, MCL1, RIPK1, TNFRS1A, BBCR3, BCL2, BIRC2, and BIRC3 were significantly reduced in patients compared with donors. Interestingly, the most marked effects were a decreased expression of BIRCs (downregulators of pro-apoptotic proteins) and an increased expression of the proapoptotic mediators BAX, BAD and caspases in the GCs of patients compared with donors. These effects were more marked in CGs than CCs. Our results suggest that low responder patients present an imbalance of apoptosis in favor of proapoptotic markers.
Limitations, reasons for caution: The main limitation of our study is the small number of replicates. The results observed in this studying using the array panels should be confirmed on a larger sample number.
Wider implications of the findings: These promising results improve the body of knowledge on low ovarian reserve pathogenesis, may aid our understanding of the physiology and regulation of apoptosis and could identify some potential microRNA biomarkers for this process. These findings could open up new therapeutic strategies for the treatment of low ovarian reserve.
Trial registration number: PI-646A.

P-609 Female carriers of reciprocal translocations tend to get adverse results in blastocyst quality and aneuploidy rate in PGD compared with male carriers

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Study question: To investigate the differences of mature oocytes number, fertilization rate, blastocyst formation rate, good blastocyst rate and aneuploidy rate in PGD between female and male carriers of reciprocal translocations.
Summary answer: Female reciprocal translocation carriers have adverse outcomes in good-quality blastocyst rate, euploidy rate and aneuploidy rate compared to male carriers.
What is known already: Present data demonstrate that there were little differences in PGD outcomes between female and male reciprocal translocation (rcp) carriers. But there are argues of limitations in sample size and in the method of aneuploidy detection by FISH.
Study design, size, duration: A retrospective study is performed to compare outcomes of PGD in female rcp carriers (n = 443) and male rcp carriers (n = 414) from 2012 to November 2015. Altogether 9173 mature oocytes were recovered and 2879 blastocysts were analyzed with Single Nucleotide Polymorphism (SNP) or Next Generation Sequencing (NGS).

Participants/materials, setting, methods: The study was performed at the Reproductive and Genetic Hospital of CITIC-Xiangya, China. Blastocysts were scored according to the standard Gardner blastocyst grading system and laser assisted trophectoderm biopsy was performed on day 5 or 6 post oocytes retrieval. Mann-Whitney-Wilcoxon (MWW) and chi-square test was used for statistical analysis.
Main results and the role of chance: Altogether 9173 mature oocytes (female rcp, $n = 4650$; male rcp, $n = 4523$) were recovered, 7807 blastocysts (female rcp, $n = 3968$; male rcp, $n = 3839$) were formed and 2879 blastocysts (female rcp, $n = 1480$; male rcp, n = 1399) were analyzed. There is no difference in age [29.5 (27, 32) vs 30 (27, 32), $p = 0.891$], number of mature oocytes [11 (7, 15) vs 12 (8, 16), $p = 0.151$], fertilization rate [85.3% (3968/4650) vs 84.9% (3839/4523), $p = 0.540$] and blastocyst formation rate [42.9% (1704/3968) vs 41.7% (1599/3839), $p = 0.248$] between female and male rcp carriers. But female rcp carriers shows an adverse result in good-quality blastocyst rate [36.2% (617/1704) vs 43% (688/1599), $p < 0.01$], euploid blastocyst rate [31% (459/1480) vs 35.2% (492/1399), $p = 0.018$], combined aneuploidy rate [26.3% (389/1480) vs 22.8% (319/1399), $p = 0.030$] compared to male rcp carriers.
Limitations, reasons for caution: The study is limited for its retrospective design. There also may exists bias caused by different operators.
Wider implications of the findings: The sample size and screening method of SNP and NGS gives us detailed informations on blastocyst formation, quality and aneuploidy rate which subsequently give us a better estimation of the chance for transferrable cycles. It'll be easier for the patients to make a decision on choosing or give-up on PGD.
Trial registration number: None.

P-610 Declined ovarian reserve is related to increased aneuploidy rate in the age group of 35–39

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Study question: To investigate aneuploidy rate between low responders (LR), normal responders (NR) and high responders (HR) in different age groups.
Summary answer: For women at the age of 35–39, aneuploidy rate increases as the recovered mature oocyte number decreases.
What is known already: Although abundant studies have shown that advanced maternal age will increase aneuploidy rate in human embryos, whether ovarian response is related to aneuploidy rate is still in debate.
Study design, size, duration: A retrospective study was performed on 1760 couples ($n = 278$ for age ≥ 40, $n = 390$ for age of 35–39, $n = 1092$ for age ≤ 34) who performed PGD from 2012 to November 2015. Aneuploidy rates not derived from maternal/paternal translocations were compared by different ovarian response (LR, mature oocyte number ≤ 5; NR, mature oocyte number = 6–13; HR, mature oocyte number ≥ 14).
Participants/materials, setting, methods: The study was completed at the Reproductive and Genetic Hospital of CITIC-Xiangya, China. Laser assisted trophectoderm biopsy was performed on day 5 or 6 post oocytes retrieval. Comprehensive chromosome screening was performed with single-nucleotide polymorphisms or next generation sequencing. Aneuploidy rates were compared with chi-square test.
Main results and the role of chance: For women over 40, aneuploidy rate between the LR, NR, HR group are not different [(75% (63/84), 78.2% (104/133) and 72.58% (45/62), $p = 0.672$)]. For women of 35–39, aneuploidy rate is statistically different between the LR, NR, HR group.[47.92% (69/144), 37.47% (178/475), 34.13% (86/252), $p = 0.022$]. For women younger than 35, aneuploidy rate between the LR, NR, HR group are also not different [25.23% (54/214), 23.63% (434/1837), 25.96% (447/1722), $p = 0.270$].
Limitations, reasons for caution: The study is limited for its retrospective design.
Wider implications of the findings: At the specific age group of 35–39, a decreased ovarian response may indicate an increase aneuploidy rate. This information will be useful for patients' decision-making in whether choose PGS or routine IVF/ICSI.
Trial registration number: None.

P-611 Cumulative oocyte vitrification improves Preimplantational Genetic Screening (PGS) clinical results in low-responder patients

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Study question: PGS result could be influenced by the low number of oocytes obtained in low-responder patients.
Summary answer: Accumulation of vitrified oocytes equates the clinical results in low-responder PGS patients when the number of collected oocytes approaches the one in normo-responder PGS patients.
What is known already: Oocyte vitrification does not increase the incidence of chromosomal abnormalities when we analyzed in a PGS cycle embryos from fresh and vitrified oocytes from the same patient.
Study design, size, duration: This retrospective observational study included 368 PGS cycles from normo-responder patients in which the origin of the oocytes of all embryos analyzed were fresh and 45 PGS cycles from low-responders patients where the origin of all analyzed embryos derived from vitrified oocytes from two or more cycles of ovarian stimulation from our PGD program (from January 2011 to December 2014).
Participants/materials, setting, methods: 377 patients were included in this study, 344 with fresh oocytes and 43 with vitrified oocytes. Oocytes were vitrified by Cryotop® method. Biopsies were performed on day 3 in Ca^{2+} and Mg^{2+} free medium (G-PGD, Vitrolife) using laser technology (OCTAX). For 24-chromosome aneuploidy screening, array-CGH was employed. Euploid embryos were transferred on day 5, supernumerary embryos were vitrified either on day 5 or day 6. Statistical comparisons were performed using a Fisher´s exact test ($p < 0.05$).
Main results and the role of chance: Mean female age was 38.9 years [IC95%: 38.6–39.3] in the fresh oocyte group and 40.9 years [IC95%: 40.1–41.7] in the vitrified group. Mean MII initials/stimulation cycle was statistically significant: 9.4[IC95%: 8.9–9.9] fresh vs 4.4 [IC95%: 6–5.2] vitrified. Mean stimulation cycles/PGS cycles was 2.2[IC95%: 1.4–2.5] in the vitrified group. No differences were found in mean MII initials/PGS cycle: 9.4 [IC95%: 8.9–9.9] fresh and 10.0 [IC95%: 8.7–11.5] vitrified, neither mean MII inyected/PGS cycle: 9.4[IC95%: 8.9–9.9] fresh and 8.8[IC95%: 7.3–10.3] vitrified. Oocyte survival rate was 88.2% [IC95%: 85.2–91.2]. Biopsied embryos/fertilized oocytes rate and D5 blastocyst rate/biopsied embryos were significantly lower in vitrified group (75.6% [IC95%: 74.3–77.3] fresh vs 69.1% [IC95%: 63.8–73.9] and 67.5% [IC95%: 65.4–69.6] fresh vs 58.1% [IC95%: 51.3–64.9] vitrified, respectively). No statistically significant differences in fertilization rate. Informativity of chromosome analysis was 98.6%. Aneuploidy rate and types of chromosomal abnormalities were similar, without statistical differences between fresh or vitrified oocytes. There was no statistical differences in efficiency rate in terms of normal D5 blastocyst/MII microinyected: 10.0% [IC95%: 9.0–11.0] vs 7.1% [IC95%: 4.6–9.6]. Ongoing pregnancy rate/transfer and ongoing implantation rate were similar in both groups (47.5%[IC95%: 40.6–54.4] fresh vs 52.6%[IC95%: 30.2–75.1] vitrified and 42.6%[IC95%: 36.5–48.1] fresh vs 42.3%[IC95%: 23.1–61.3] vitrified, respectively).
Limitations, reasons for caution: One limitation of the study is the lower number of cases included in the second group. Another limitation is population differences in terms of age and infertility aetiology, but despite these differences and the poor prognosis expected in the low responder group, the results are encouraging.
Wider implications of the findings: Accumulation of vitrified oocytes before the actual PGS cycle is a possible strategy that might increase patient's chances for a healthy pregnancy, matching the results of patients of low responder to normo-responders. Increasing the oocytes cohort contributes to significantly lower the dropout rate and the embryo transfer cancellation.
Trial registration number: None.

P-612 Aneuploidy vs Age: A study chromosome by chromosome

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Study question: Aneuploidy increases with maternal age, but are there specific chromosomes that are more prone to aneuploidy than others?
Summary answer: Aneuploidy for chromosomes 13, 15, 16, 19, 21, and 22 is extremely correlated by age, but for chromosome 1 and 3 is independent of age.
What is known already: The association between maternal age and aneuploidy has been established since the 1990s (Sherman et al., 1994; Phillips et al., 1995; Munne et al., 1995). Early FISH studies established correlation between aneuploidy and age for chromosomes X, 16, and 21 (Hassold et al., 1993; Munne et al., 1995) and other chromosomes (Bahçe et al, 1999). Success rates of in vitro fertilization have been enhanced by implementing routine comprehensive chromosome screening (CCS) as shown by recent randomized trials. These techniques, mainly aCGH and NGS, are able to analyze all 24 chromosomes expanding the reach of the few chromosomes FISH could ever analyze.
Study design, size, duration: Retrospective study of 56,328 embryos at blastocyst stage from 11,272 cycles from 156 centers across the USA from January 2014 to August 2015.
Participants/materials, setting, methods: 48048 embryos were processed by aCGH (85.3%), and 8280 were processed by NGS (15.7%). Embryos were distributed in age groups according to the START guidelines.
Main results and the role of chance: The aneuploidy information of every chromosome in every age group was noted. The correlation between the age and the aneuploidy was calculated by the Pearson's correlation coefficient, and the *p* value for Pearson was calculated to ascertain if this correlation was significant and to what degree (Table I). Although the majority of the chromosomes showed correlation with age (except for chromosomes 1 and 3), some of the chromosomes showed a higher degree of correlation than others (13, 15, 16, 19, 21, and 22). The EGD group is quite homogeneous in regards to aneuploidy, each chromosome has more or less the same rate of aneuploidy. But as the maternal age of the groups increases, the aneuploidy rate for some chromosomes increases faster than other.

Chromosome	Correlation (R)	Pearson value	Extremely significant (0.01)
13	0.9278	0.0076	yes
15	0.9288	0.0074	yes
16	0.9815	0.0005	yes
19	0.9390	0.0055	yes
21	0.9567	0.0028	yes
22	0.9809	0.0005	yes

Limitations, reasons for caution: The comparison between both CCR techniques will be discussed during the presentation. Embryo survival at blastocyst stage may bias the preponderance of the aneuploidy of some.
Wider implications of the findings: There is a relation between size of the chromosome and aneuploidy. The chromosomes with smaller size have an increased aneuploidy rate. Additionally the egg donor group (EGD) has a more homogeneous aneuploidy rate among all the chromosomes. These two facts may point out that female meiotic errors affect smaller chromosomes.
Trial registration number: N/A.

P-613 Trophectoderm biopsy and transfer in a subsequent frozen thaw embryo replacement cycle in preimplantation genetic diagnosis cycles

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Study question: What is the pregnancy outcome of trophectoderm biopsy and transfer in a subsequent frozen embryo replacement (FER) cycle in couples undergoing preimplantation genetic diagnosis (PGD)?
Summary answer: In PGD cycles, trophectoderm biopsy and transfer in FER cycles offers advantageous pregnancy outcomes compared with cleavage stage biopsy.
What is known already: Traditionally, embryo biopsies for PGD cycles have been performed at cleavage stage, with fresh transfer of a suitable blastocyst. ESHRE PGD Consortium collection I-XII data showed 92% and 0.7% were cleavage and blastocyst stage, respectively. Blastocyst biopsy compared with cleavage stage biopsy has been reported to have a higher rate of genotyping, less amplification failure, less allele drop out and fewer embryos to biopsy. It is also associated with higher implantation and pregnancy rates than cleavage stage biopsy. Furthermore, FER can prevent late onset ovarian hyperstimulation syndrome (OHSS), is associated with improved perinatal outcomes and a higher ongoing pregnancy rate.
Study design, size, duration: This is a retrospective case series of PGD cycles performed from July 2014 to December 2015. 85 couples had biopsy at blastocyst stage and vitrification. 66/85 couples had a suitable embryo to transfer. Of the remaining 19 couples, 12 are awaiting FER at the time of writing, whilst 7 couples had blastocysts which were not suitable for transfer.
Participants/materials, setting, methods: Couples were either referred from a regional genetic centre, or self-referred. Couples were counselled and standard IVF protocols were applied. Embryos were cultured to blastocyst and biopsied as described by Gardner, 2007. The cells were sent to the reference laboratory for genotyping. Standard procedures for thaw of embryos were performed and blastocysts were transferred in a subsequent medicated FER cycle. Patients carried out their urinary pregnancy test 16 days later.
Main results and the role of chance: 85 cycles of trophectoderm biopsy and vitrification were performed. 72/85 (85%) were for single gene disorders and 13/85 (15%) for chromosomal rearrangements. A total of 398 blastocysts were biopsied. 182/398 (48%) blastocysts were suitable for transfer. One blastocyst required repeat thaw and biopsy, due to initial amplification failure, which resulted in a suitable embryo to transfer. None of the blastocysts failed to survive the thaw process on the day of FER. All couples had elective single embryo transfer.

Of 54 PGD cycles for single gene disorder with known pregnancy outcome, 42 (78%) FER cycles resulted in a positive pregnancy test (95% CI 65–87). The ongoing pregnancy rate is 38/54 (70%) (95% CI 57–81). 2/54 (4%) FER cycles were biochemical pregnancies (95% CI 1–13) and 2/54 (4%) resulted in first trimester miscarriage (95% CI 1–13). 12/54 (22%) FER cycles had a negative pregnancy test (95% CI 13–35).

Of 12 PGD cycles for chromosomal rearrangements with known pregnancy outcome, 9 (75%) FER cycles resulted in a positive pregnancy test (95% CI 47–91). The ongoing pregnancy rate is 7/12 (58%) (95% CI 32–81). 2/12 (17%) such cycles resulted in first trimester miscarriage (95% CI 5–45).
Limitations, reasons for caution: This is the largest number of pregnancy outcomes so far reported from a trophectoderm biopsy, vitrification and FER dataset for single gene disorders and chromosomal rearrangements. However, results are retrospective and non-randomized.
Wider implications of the findings: Trophectoderm biopsy, vitrification and FER can be successfully applied to PGD cycles for single gene disorders and chromosomal rearrangements. This approach increases the rate of genotyping and moreover, implantation and pregnancy rates exceed those following cleavage stage biopsy.
Trial registration number: 0.

P-614 Number of blastocysts biopsied as a predictive indicator for obtaining at least one normal/balanced embryo with single nucleotide polymorphism microarray in translocation cases

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Study question: How many number of blastocysts to be biopsied is optimum, in order to predict at least one normal/balanced blastocyst after SNP-PGD for translocation carriers?
Summary answer: 3 and 4 accumulated blastocysts were predicted for Robertsonian and reciprocal translocation carriers younger than 37 with basal FSH level under 10.2 IU/L respectively.
What is known already: For a RT carrier, depending on segregation patterns in meiotic divisions, only 1/3 normal and 1/3 balanced karyotype gametes are alternate and further, can produce normal or RT karyotype embryos. As for a

rcp carrier, the alternate gametes are merely account for 1/9. Blastocyst-stage biopsy rather than cleavage-stage biopsy is now generally recommended in the advantage of lower mosaic rates and less biopsy-related damages. WGA-based SNP microarrays for PGD have the capability to screen concurrent 24 chromosomes in addition to testing for the specific unbalanced chromosome complement expected arising out of the parental translocation.

Study design, size, duration: Blinded retrospective study from July 2013 to December 2014, including 51 RT couples who underwent 55 cycles of PGD and 131 rcp pairs who underwent 181 cycles.

Participants/materials, setting, methods: Couples recruited were for the indication of only one partner carried translocation. Trophectoderm biopsy on day 5 or 6 followed by WGA-based SNP microarrays was performed in a Hospital-based IVF center. Main outcome measures were balanced/normal embryo rate (transferrable rate) and clinical pregnancy rate.

Main results and the role of chance: Reliable SNP-PGD results were obtained for 355 out of 379 (93.7%) biopsied blastocysts in Robertsonian group and 986 out of 1053 (93.6%) in Reciprocal group. The average numbers of biopsied embryos per patient and normal/balanced embryos per patient, the mean balanced/normal embryo rate per patient and the rate's 95% confidence interval (CI) were 7.4, 3.1, 40.7%, 34.6%—46.9%, respectively, in Robertsonian translocation carriers, and 8.0, 2.1, 27.3%, 23.9%—30.7%, respectively, in reciprocal translocation carriers. In adjusted linear regression model, the only three significant factors affecting the number of genetically transferrable embryos were the number of biopsied embryos (coefficient: 0.489, $p = 0.001$), basal FSH level (coefficient: 0.216, $p = 0.023$) and maternal age (coefficient: 0.060, $p = 0.042$). ROC analysis was then performed in a subgroup including female under age 37 and basal FSH level below 10.2 IU/L. Using ROC, a cut-off level of 2.5 for the number of biopsied embryos when Robertsonian translocation carriers intended to obtain at least one balanced/normal embryos (sensitivity = 86.8%, specificity = 66.7%, AUC = 0.912), and a 3.5 cut-off level for reciprocal translocation carriers (sensitivity = 90.4%, specificity = 73.3%, AUC = 0.877) were established. A 44.2% and 42.6% clinical pregnancy rate per ET in RT group and in rcp group was respectively achieved ($p = 0.836$).

Limitations, reasons for caution: Distinguishing a balanced embryo from a normal one was still inevitable. A specific abnormal product would not be scored if small copy number variants or polymorphisms were under 5MB cut-off value due to limited detection rate of SNP array.

Wider implications of the findings: The number of transferrable embryos could be roughly predicted. Besides, for carriers who need a series of stimulation cycles to accumulate embryos, recommendation about when to suspend COH for biopsy could be given. As for carriers with high OHSS risk, the initial does of Gn could be compromised.

Trial registration number: This study was approved by the Ethics Committees of both affiliated hospitals of Sun Yat-sen University. Written consent was obtained from each participant.

P-615 Female chromosomal structural abnormalities do not influence the ovarian response in controlled ovarian stimulation

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Study question: To determine whether patients of chromosomal structural abnormalities have an impact on ovarian response to controlled ovarian stimulation (COS) in preimplantation genetic diagnosis (PGD) patients.

Summary answer: This largest cohort of chromosomal structural abnormalities patients treated with PGD showed that ovarian response to COS was not impaired.

What is known already: In female translocation carriers, balanced X:autosomal translocations are described as being associated with gonadal dysfunction and premature ovarian failure. Some cases of balanced autosomal Roberstonian and reciprocal translocations associated with premature ovarian failure have been reported Few studies concluded that impaired ovarian function resulted in female translocation carriers. Only one study in 2005 concluded that there was a higher proportion of low responders in female translocation carriers Dechanet et al. report the largest study in 2011 which suggest that female chromosomal structural abnormalities did not influence the results of COS in PGD.

Study design, size, duration: A retrospective analysis was performed in a single IVF center. All couples for chromosomal structural abnormalities include Roberstonian translocations, reciprocal translocations and chromosomal inversion, presenting to PGD in either partner from 2013 through 2015. A total of 1075 cycles in 892 women were completed. Stimulation and embryology outcomes were compared between two groups: 559 cycles in 457 women with chromosomal structural abnormalities compared to 516 cycles in 435 women whose male partner had a chromosomal structural abnormalities.

Participants/materials, setting, methods: Average female age, day 3 Follicle stimulating hormone (FSH), antral follicle count (AFC), Anti-Müllerian hormone (AMH) were similar in both groups. Statistical analysis was performed comparing stimulation protocols, oocyet retrival and other cycle parameters.

Main results and the role of chance: 559 cycles of PGD from 457 female chromosomal structural abnormalities were compared to 516 cycles from 435 patients whose male partner had a chromosomal structural abnormalities. No difference was observed for patients characteristics: age (29 years and 28 years, $p = 0.281$), FSH (6.13IU/L vs 6.0 IU/L, $p = 0.40$), AMH (3.9 ng/mL vs 4.83 ng/mL, $p = 0.134$) and AFC (16 vs 17, $p = 0.408$). Concerning COS parameters, no difference was found for the duration of stimulation (11 days vs 11 days, $p = 0.052$), total dose of rFSH (1987.5IU vs 1886.25 IU, $p = 0.08$). No difference was shown for the number of retrieved oocytes (12 vs 13, $p = 0.307$). The cancellation rate for no embryos for transfer after SNP-array was different (13.4% vs 8.5%, $p = 0.01$).

Limitations, reasons for caution: Previous studies have shown a high dose of FSH may be associated with high chromosomal aneuploidy. We need further randomized controlled study to prove whether the cancellation rate for no euploid embryos after SNP-array was different is caused by the increased women age or the mild increased dosage of gonadotropin.

Wider implications of the findings: Our study showed that ovarian response of female chromosomal structural abnormalities was not impaired. Using a standard dose of stimulus allowed an adequate ovarian response.

Trial registration number: none.

P-616 Investigation of the relationship between telomere length and pregnancy outcome

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Study question: Is telomere length in human blastocysts potentially used as a predictive marker for pregnancy outcome?

Summary answer: Telomere length in human blastocysts is associated with the pregnancy outcome.

What is known already: Telomeres are the end of eukaryotic chromosome which participate in protecting the chromosome from degradation and maintain chromosome stability. Recent studies have shown that telomeres length is associated with embryo aneuploidy and development. Telomere length in blastocyst stage is longer than cleavage stage. The telomere length in oocyte was investigated, a shorter length was found in non-pregnancy woman as compare to pregnancy woman. Little is known regarding the correlation between telomere length in human blastocysts and pregnancy outcome.

Study design, size, duration: This study was retrospective by including our patients who were subjected to preimplantation genetic screening during January – June 2015. Chromosome screening by next-generation sequencing and bioinformatics analysis for telomere length were performed. Fifty-two blastocysts were biopsied either day 5 or 6. All embryos were transferred and clinical outcome were monitored. Data were collected from only single embryo transferred (SET) cases. Outcome of pregnancy and non-pregnancy cases were compared.

Participants/materials, setting, methods: Telomere lengths of biopsied blastocysts were assessed using next-generation sequencing with bioinformatics analysis to evaluate telomere length in all arms of chromosome (45 regions) of pregnant and non-pregnant groups.

Main results and the role of chance: A significant correlation between telomere length and pregnancy outcome was found in many chromosome arms including 1p, 11p, 11q, 12p, 17p, 20p ($p < 0.05$). Interestingly, the majority of blastocysts of successful pregnancy (35/45) result showed longer telomere length compared to unsuccessful pregnancy (10/45).
Limitations, reasons for caution: We detected telomere length only. Other factors such as chromosome regions other than telomere, etc. may interfere with this study.
Wider implications of the findings: In this study, we performed next-generation sequencing with bioinformatics analysis for determination of telomere length in order to improve the effectiveness of embryo selection.
Trial registration number: Research Involving Human Research Participant, Health Science Group, Chulalongkorn University (RECCU), Thailand (COA 162/2015).

P-617 Dynamics of Eukaryotic Translation Initiation Factor 4 and p70S6K signaling during human primordial-to-primary oocyte transition

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Study question: What are the transcriptional dynamics of the Eukaryotic Translation Initiation Factor 4 (eIF4) and p70S6 Kinase signaling during human oocyte activation?
Summary answer: Several members of the eIF4 and p70S6K signaling pathway were significantly ($p < 0.05$) up-regulated during human primordial-to-primary transition, including the mTOR pathway effector *EIF4E*.
What is known already: Correct molecular governance of the human primordial-to-primary oocyte transition is a prerequisite for maintaining a sufficient oocyte stock to last a reproductive lifespan as well as ovulating fertilizable competent eggs. Among others, the PI3K/AKT-, and mTOR signaling pathways regulates primordial oocyte dormancy-, and activation, working both independently, and in parallel. Downstream effectors of these pathways are p70S6 Kinase, involved in cell growth, and The Eukaryotic Translation Initiation Factor 4 with members; eIF4A-, B, E, and γ, interacting as a complex that recruits mRNA to ribosomes initiating translation. Knowledge on eIF4, and p70S6K signaling in the primordial-to-primary transition is spares.
Study design, size, duration: We performed transcriptome analysis of pure pools of isolated human primordial ($n = 436$), and primary ($n = 182$) oocytes, respectively, from three normo-ovulatory patients in their late 20s via Next Generation Sequencing. Following quality control, and normalization, transcripts significantly ($p < 0.05$) highly expressed (Fragments Per Kilobase Of Exon Per Million Fragments Mapped - FPKM value) and with significant ($p < 0.05$) concordance in FPKM level between the three patients were subjected to enrichment analysis.
Participants/materials, setting, methods: Pure pools of primordial, and primary oocytes from three normo-ovulatory patients were isolated using Laser Capture Microdissection. Following transcriptome sequencing on the Illumina HiSeq platform, significantly ($p < 0.05$) highly expressed genes in primordial, and primary oocytes, respectively, and significantly ($p < 0.05$) differentially expressed genes between the two oocyte stages were subjected to enrichment analysis using Ingenuity® Pathway Analysis (IPA). For this study, we analyzed protein-coding transcripts only.
Main results and the role of chance: In the primordial oocyte, 38 highly expressed genes were assigned "Regulation of eIF4 and p70S6K signaling" which was significant ($p = 3.62E-16$). These included the *eIF4EBP2,* a transcript coding for a member of the EIF4E binding family of proteins that negatively regulate the EIF4E complex, and thus translation. Also, *EIF4G* was significantly highly enriched in primordial oocytes. EIF4G associates with EIF4E to form the functional translation complex EIF4F, however, *EIF4E* was *not* highly expressed in primordial oocytes. In the primary oocyte, 44 highly expressed genes were assigned "Regulation of eIF4 and p70S6K signaling" which was significant ($p = 4.76E-22$). These included *EIF4E*, *EIF4G, EIF4H*, *PAIP1*, and *PIK3CB*. No members of the EIF4E binding family were highly expressed in primary oocytes. When looking at genes differentially expressed in oocyte from the two stages, genes significantly up-regulated from primordial-, to primary oocytes were, among others, *EIF4E* and *PIK3CB*. Transcripts significant down-regulated from primordial- to primary included *EIF3D*, coding for a member of a complex that binds to the 40S ribosome and helps maintain the 40S and 60S ribosomal subunits dissociated and thus inactive in translation.
Limitations, reasons for caution: Tissue from a limited number of patients were included. This limitation was met by isolating large quantities of pure oocytes from each stage, sequencing each stage from each patient separately, and only including transcripts with low variance between three patients as well as high FPKM quantity in the enrichment analysis.
Wider implications of the findings: These results indicate a far more translational active primary oocyte as compared to the primordial oocyte and that "Regulation of eIF4 and p70S6K signaling" seems to have a central role in regulating translation of stored mRNA during the primordial-to-primary oocyte transition.
Trial registration number: Not a clinical trial.

P-618 Sperm non-coding RNA profiling as an indicator of reproductive potential

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Study question: We question if sperm non-coding RNA have an influential role in profiling the infertile male partner and predict his ability to achieve a viable pregnancy.
Summary answer: Non-coding RNA (ncRNA) contributed by the spermatozoon at the time of fertilization are paramount regulatory molecules that can affect embryo developmental competence.
What is known already: Standard semen analysis has many shortcomings when providing information on the performance of spermatozoa when used for assisted reproductive technologies (ART). In men with idiopathic infertility, supplementary tests may be pivotal to gain insight on the paternal contribution to the zygotic genome. NcRNAs are functional RNA molecules that are transcribed from DNA but not translated into proteins. RNA profiling of the spermatozoon unveils a large variety of ncRNA that are now believed to play a very important role in regulating spermatogenesis, fertilization, embryo development and transgenerational epigenetic inheritance.
Study design, size, duration: In a 12-month period, we assessed the RNA profile of men undergoing infertility screening with specific focus on ncRNA. The analysis was performed by measuring the abundance of small non-coding (sncRNA) and long non-coding RNA (lncRNA) and comparing to semen characteristics and ART outcome. RNA extraction from 26 semen specimens was carried out on men undergoing infertility screening, with 19 men in the study group and that were compared to 7 fertile.
Participants/materials, setting, methods: An average of 25×10^6 human spermatozoa was used to isolate total RNA using a spin column commercial kit. The nucleic acid quality and spermatozoal RNA concentration was assessed. The RNA samples were then made into paired-end libraries. Pilot paired-end 76bp RNA-Sequencing (RNA-Seq) using an Illumina platform (NextSeq 500) was carried out and expanded to 60M reads. Expression values were calculated in Fragments Per Kilobase Of Exon Per Million Fragments Mapped (FPKM) and normalized read counts.
Main results and the role of chance: Of the 26 men screened, 8 were selected for sperm RNA-Seq with an average age of 26 ± 5 years presenting with a sperm concentration of 27.3 ± 27, and motility of 46.6 ± 24, and morphology of 3.0 ± 2. From the 23,261 genes assessed, statistical analysis evidenced 28 (0.12%) lncRNAs that were differently expressed ($p < 0.0001$) between the cohort of men all capable to naturally conceive ($n = 3$, control cohort) and men unable to sustain a pregnancy ($n = 5$, study cohort), even after intracytoplasmic sperm injection (ICSI) treatment. Over 90% of these identified genes were overexpressed in the infertile group when compared to the control. All 28 differentially expressed RNAs were classified as long intergenic non-coding RNA (lincRNA). Additionally, a set of genes ($n = 16$) had complete lack of expression

in the cohort of men that were unable to conceive. In relation to their function, 11/16 (68.8%) genes are considered a) to guide chemical modification of other RNAs, b) are associated with methylation, or c) affect both stability and translation of messenger RNA (mRNA).
Limitations, reasons for caution: This study investigates the regulatory role of ncRNA on embryo developmental competence and needs to be confirmed in a larger cohort. The exact regulatory function and localization within the spermatozoon, together with the resulting role in the embryonic genome need to be further investigated.
Wider implications of the findings: Profiling men seeking infertility treatment via RNA-Seq to supplement standard semen analysis may aid in the diagnosis and management of these couples. Screening men for an epigenetic imbalance of sncRNA and lncRNA provides crucial information on the etiology of idiopathic infertility and overall reproductive capacity of the infertile male.
Trial registration number: N/A.

P-619 Alteration of epigenetic profile in cumulus cells of polycystic ovary syndrome patients

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Study question: This study aimed to evaluate global histone methylation and acetylation levels of cumulus cells in infertile patients with polycystic ovary syndrome (PCOS).
Summary answer: Differential methylation and acetylation levels of lysine 9 of histone3 (H3K9me2 and H3K9ac) in cumulus cells was observed in PCOS patients compared to non-PCOS women.
What is known already: PCOS is the most common endocrine disorder in infertile women. Differential genetic and epigenetic pathways of several genes in various tissue and cell types have been identified as important in etiopathogenesis of PCOS. Previous studies showed that changes in genes expression of CCs which surround oocyte are associated with pathogenesis of PCOS. Of essential genes for ovarian functions and folliculogenesis is CYP19A1 (aromatase coding gene) and its involvement in the pathogenesis of PCOS has been studied. However, comprehensive epigenetic studies in cumulus cells especially for aromatase aberrant gene expression in such disorder have been lacking till date.
Study design, size, duration: Case–control study was conducted on 24 patients (12 infertile PCOS patients and 12 patients with tubal factors of infertility or egg donor) aged 18–36 year old, who underwent ovarian stimulation with GnRH antagonist for Intracytoplasmic Sperm Injection (ICSI) between November 2014 to April 2015. Informed consents were obtained from the participants. Cumulus oocyte complexes (COC) were obtained from follicles during ovarian puncture. Shortly before ICSI, CCs were stripped from the COC with hyaluronidase.
Participants/materials, setting, methods: In order to identify global occupancy level of histone modifications marks (H3K9me2/H3K9ac) to chromatin fractions of CCs, chromatin from samples was extracted and Nucleosome-ELISA was performed using H3K9 acetylation/methylation antibodies. Also, RNA extraction and cDNA synthesis were performed. Expression of CYP19A1 gene was examined by qRT-PCR. DNA incorporation of MeCP2 (as a marker of DNA methylation) and histone modifications marks in PII, PI.3 and PI.4 promoters of CYP19A1 gene were examined by ChIP-Real time-PCR assay.
Main results and the role of chance: Data demonstrated significant increase in global occupancy levels of H3K9 acetylation mark and also significant decrease of H3K9 methylation mark to regulatory regions of chromatin in cumulus cells of PCOS patients vs control group. Also, relative expression of CYP19A1 gene was significantly higher in CCs from PCOS patients compared with the control group. In CCs of PCOS patients, incorporation of histone H3K9ac mark in PII, PI.3 and PI.4 promoter regions of CYP19A1 were significantly higher than those of control group ($p < 0.05$). Furthermore, a significant hypomethylation at H3K9 of promoter PII was observed in PCOS patients ($p = 0.0001$), whereas no significant difference of H3K9 methylation level was detected in PI.3 and PI.4 promoters between patients and control groups. Furthermore, PII and PI.3 promoters in CCs of PCOS patients were significantly DNA hypomethylated in compare to controls ($p = 0.003$ and $p = 0.001$, respectively).
Limitations, reasons for caution: Further studies are necessary to evaluate the role of transcription factors and to elucidate the precise mechanisms involve in aromatase gene expression in PCOS patient.
Wider implications of the findings: Epigenetic alterations could change the genes expression in CCs, which may play a key role in the abnormal folliculogenesis and ovarian function in PCOS. Mechanism of changes in aromatase gene expression, parallel to epigenetic alterations may help to further understand the ovarian hyperstimulation syndrome (OHSS) and its management in these patients.
Trial registration number: N/A.

P-620 Male age is not related with high rates of spermatozoa and embryo aneuploidy

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Study question: Is male age related with high aneuploidies in sperm and embryos?
Summary answer: Increased paternal age is not associated with sperm and embryos aneuploidies, therefore aging men do not have increased risk of producing chromosomally abnormal offspring.
What is known already: The frequency of IVF embryo aneuploidies is estimated to be high and its occurrence is related to maternal and paternal factors. The main factor is female age, however paternal factor could play an important role, mainly in patients with abnormal seminal parameters and chromosomal segregation. Nevertheless, the effect of male aging in aneuploidies remains controversial. Since the introduction of new PGS techniques more data have been added to elucidate the male age factor effect on aneuploidies. Moreover, to the best of our knowledge nothing is known about the effect of male aging and aneuploidies using PGSv2.0 in egg-donor cycles.
Study design, size, duration: We performed a retrospective observational study (from January 2013 to December 2015). To show the relationship between male age and sperm aneuploidies, 428 sperm FISH analysis were included in the study. We analysed 155 blastocysts from 51 oocyte-donor cycles in which the partner had a normal sperm FISH in order to evaluate the effect of male age in embryo aneuploidies avoiding the confounding effect of female factor and the abnormal FISH in the embryo aneuploidy.
Participants/materials, setting, methods: Sperm FISH (7 chromosomes analysed) was performed to patients who attended Instituto Bernabeu with a previous clinical history of repetitive miscarriage, implantation failure or severe male factor. Moreover, we included couples attended Instituto Bernabeu for oocyte donation where PGS was performed for previous miscarriage or implantation failure. For PGSv2.0 trophectoderm genome was amplified and aCGH performed using Agilent SurePrintG3 8 × 60K. The association between variables and male age was evaluated by logistic regression and chi-square (SPSSv20.0).
Main results and the role of chance: The seminal parameters and sperm FISH from 428 patients (from 20 to 53 years old) were evaluated. 31% of the analysed patients had an abnormal sperm FISH. No significant difference was reported according to male age and semen parameters according to WHO 2010 criteria ($p = 0.273$). Moreover, no differences in the results of the basic semen parameters and abnormal sperm FISH ($p = 0.626$) were reported. Finally, no differences were reported for male age and abnormal FISH ($p = 0.166$), however patients older than 50 y showed a tendency to higher sperm aneuploidies (50.0% vs 30.5%, $p = 0.081$). To show the effect on embryo aneuploidy we analysed 155 blastocyst from 51 oocytes from donor cycles where the male partner had a normal sperm FISH. The embryo aneuploidy rate was 26%. In these cycles no significant difference was reported between embryo aneuploidy and seminal parameters ($p = 0.83$). When the data was analysed according to sperm morphology and motility, astenozoospermia (46.2%) and teratozoospermia

(44%) showed higher aneuploidies rates than normozoospermia (24.6%) without statistical significance ($p = 0.14$). According to male age no differences were reported in embryo aneuploidy rate ($p = 0.787$) in oocyte donors where the partner had normal FISH.

Limitations, reasons for caution: The study is limited by its retrospective nature. A higher sample size or a prospective randomized design should be used in future studies to corroborate the current findings. In addition, research into the contribution of non-genomic paternal factor in the offspring of older male is needed.

Wider implications of the findings: This investigation reveals that, in cycles controlled for female age and partner with normal sperm FISH, increased paternal age is not associated with embryo aneuploidy. Moreover, this finding has been showed in sperm FISH studies.

Trial registration number: No trial.

P-621 Non-Invasive Prenatal Testing uptake and results among IVF patients in Ontario, Canada: preliminary results

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Study question: To assess the uptake and results of Non-Invasive Prenatal Testing (NIPT) among patients who conceived through the use of in vitro fertilization (IVF).

Summary answer: This study found that the uptake of NIPT in Ontario, Canada among women who conceived with IVF and/or frozen embryo transfer (FET) was 6.17%.

What is known already: NIPT analyzes cell-free fetal DNA in the plasma of pregnant women to detect fetal trisomies. In 2008, two groups used massively parallel sequencing to identify the over-representation of chromosome 21 in the maternal plasma of pregnancies affected with Down syndrome. Clinical validation trials showed high sensitivity and specificity for detecting common fetal chromosome disorders; subsequently this test became available in Ontario to women who met the risk criteria or on a self-pay basis. Previous studies among IVF conceptions showed that first and second trimester serum markers are different; therefore NIPT may be more appropriate for screening women who used IVF.

Study design, size, duration: This retrospective cohort study included 9,238 IVF and FET treatment cycles conducted in 2013 and 5,147 NIPT screens performed in 2013 and 2014. A proprietary probabilistic linking algorithm developed to link multiple data sources was used to link the NIPT records to the birth records in the Better Outcomes Registry & Network (BORN) Ontario database. This linking algorithm incorporated demographics, health card number, chart number, birthdate, estimated date-of-birth and LMPs.

Participants/materials, setting, methods: The analysis was conducted with data from BORN Ontario and the Canadian Assisted Reproductive Technologies Register (CARTR) Plus. BORN Ontario captures all births within the province of Ontario and CARTR Plus contains all fertility treatment cycles in Canada. This study assessed all IVF and FET cycles in Ontario, Canada that were performed from January 1 through December 31, 2013.

Main results and the role of chance: Among patients who used IVF or FET to conceive, there were 156 ongoing clinical pregnancies that had NIPT to screen for aneuploidies. In comparison, there were 4,988 pregnancies screened with NIPT who conceived spontaneously. NIPT uptake was 6.17% among patients who used IVF to conceive. The majority of women who had IVF and NIPT were between 35 and 39 years of age (55.4%), while the remainder were <35 years (25.2%) and ≥40 years (19.5%). These preliminary results found no high risk cases of Trisomy 18, XO, XXX or XXY among the IVF cohort and less than 6 high risk cases of Trisomy 21 and Trisomy 13 were identified. In comparison, the spontaneous conception group had a similar proportion of high risk Trisomy 21 cases, but a smaller proportion of Trisomy 13 high risk cases. Additionally, there were 109 cases identified as IVF conceptions on the NIPT record that did not link to an IVF record in CARTR Plus, which is considered as the gold standard for Canadian fertility data.

Limitations, reasons for caution: This was a preliminary analysis. This study contains the majority of NIPT records; however there are three additional labs conducting NIPT screening that have not been included yet, as their data were not available at the time of the study.

Wider implications of the findings: Once data for all Ontario-based NIPT screening has been analyzed we will be able to conclude whether NIPT identifies a greater association between IVF and the risk of aneuploidies compared to the risk for women who conceive spontaneously.

Trial registration number: N/A.

P-622 Intercycle variation in embryo aneuploidy rates

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Study question: Should we expect similar aneuploidy rates among different cycles in the same couple?

Summary answer: The embryo aneuploidy rate from the first CCS cycle is unable to predict the embryo aneuploidy rate in the next CCS of the same couple.

What is known already: The development of CCS has offered valuable insight into the chromosomal status of embryos. Embryo chromosomal aneuploidy is the most common cause of unsuccessful pregnancy after IVF. CCS applied as a therapeutic tool could improve implantation and live birth rates. Embryo aneuploidies occurrence is related to maternal and paternal factors. Some predictive factors upon embryo aneuploidy rate such as female age have been identified. Nevertheless, the recurrence of aneuploidy with repeated attempts and the predictive value of subsequent outcome remains unclear. Moreover, to the best of our knowledge nothing is known about this topic using PGSv2.0.

Study design, size, duration: We performed a retrospective observational study between January 2014 and December 2015 in Instituto Bernabeu, Alicante, Spain. The study includes the data analysis of 222 trophectoderm biopsies from blastocysts with conclusive CCS results obtained from 76 CCS cycles performed by 38 couples. Couples were categorized into two groups according to aneuploidy rate: below 33%, good prognosis; over 33%, bad prognosis.

Participants/materials, setting, methods: PGS was performed to couples who attended with a previous clinical history of recurrent miscarriage, repeat implantation failure or severe male factor. For PGSv2.0 trophectoderm genome was amplified and aCGH performed using Agilent SurePrintG3 8 × 60 K. The results from the two consecutive cycles performed by the same couple were compared in pairs for each couple. The association between variables was evaluated by Wilcoxon test for paired samples (SPSSv20.0).

Main results and the role of chance: Results from CCS were obtained in 97.5% of the biopsied embryos (5/222). To summarize, in the first and second CCS cycles the average maternal age (35.4 vs 35.5), the number of retrieved oocytes (13.21 vs 15.18), the number of the mature oocytes (11.39 vs 12.21) and the average of biopsied embryo (2.97 vs 2.87) showed no significant differences between cycles ($p > 0.05$). However, when we compared the embryo aneuploidy rate between the first CCS cycle and the second CCS cycle from the same couple, significant differences were reported ($p = 0.011$). According to the categorized (good and poor prognosis) groups, where 33% of embryo aneuploidy rate was used as cut-off, positive and negative predictive values of the first CCS cycle predicting the second CCS cycle were calculated. The positive predictive value was 61% and the negative predictive value was 57%. The sensitivity and specificity of the first CCS cycle predicting the second was 86.3% and 25% respectively.

Limitations, reasons for caution: A higher sample size should be used in future studies to corroborate the current findings. Different categories according to the number of biopsied embryos in each cycle could help us to clarify this finding.

Wider implications of the findings: Our data shows that patients with high embryo aneuploidy rate in the first CCS are highly likely to have a different rate in their second cycle. These data should help patients and clinicians after an unsuccessful CCS to make a decision whether to continue with their own or donated gametes.
Trial registration number:

P-623 Decreased number of mature oocytes in BRCA1 mutation carriers in IVF/PGD.

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Study question: Do healthy *BRCA1/2* carriers produce fewer mature oocytes after ovarian hyperstimulation for IVF with preimplantation genetic diagnosis (PGD), when compared to a PGD control group?
Summary answer: Ovarian response, expressed as the number of mature oocytes, was reduced in *BRCA1* carriers, but not in *BRCA2* carriers.
What is known already: The *BRCA* genes are involved in DNA double stranded break repair. It was hypothesized that an accumulation of DNA damage, resulting from an impaired *BRCA* function, would not only lead to a higher risk of ovarian cancer, but might also affect ovarian reserve. Several studies have assessed ovarian reserve in *BRCA1/2* carriers using different endpoints (e.g., anti-Müllerian hormone, age of natural menopause). Conflicting results have been published, also in two studies reporting on ovarian response to hyperstimulation for IVF (/PGD). Besides, the latter studies were of relatively small size and the majority of *BRCA* carriers included were cancer patients undergoing fertility preservation.
Study design, size, duration: A retrospective, multicenter cohort study was performed with data of PGD treatments performed from January 2006 until September 2015. With 50 *BRCA1/2* carriers available, we obtained a power of 80% (α 0.05) to detect a difference of the previously described magnitude (i.e., 3.4 oocytes) when including 200 controls.
Participants/materials, setting, methods: Healthy female *BRCA1/2* carriers, without a history of breast cancer and/or chemotherapy, who underwent PGD were included in the exposed group. Controls underwent PGD for other PCR-PGD indications, unsuspected for a possible diminished ovarian reserve status. Only the first cycle performed in a long GnRH suppressive agonist protocol with at least 150 IU follicle stimulating hormone (FSH) was included for each couple.
Main results and the role of chance: Of 50 exposed couples, 38 were stimulated in a long agonist protocol with at least 150 IU FSH during their first cycle. This was the case for 154 out of 200 control couples. The mean number of mature oocytes was 6.6 ± 3.3 in *BRCA1* carriers, 7.4 ± 3.3 in *BRCA2* carriers, and 8.9 ± 4.7 in controls. Multiple linear regression analysis with the number of mature oocytes as dependent variable and adjustment for center, age, BMI, type of gonadotropin used and the total dose of gonadotropins administered, revealed a significantly lower yield in *BRCA1* mutation carriers as compared to controls (p = 0.01), but not in *BRCA2* mutation carriers as compared to controls (p = 0.50).
Limitations, reasons for caution: The exclusion of couples who were treated in another IVF protocol or with less than 150 IU FSH, may have introduced selection bias. Furthermore, we were unable to control for potential confounding lifestyle factors (e.g., smoking).
Wider implications of the findings: Our findings may illustrate an influence of the *BRCA1* gene on ovarian reserve. Prospective studies are needed to provide final proof for the role of the *BRCA* genes in ovarian reserve conditions.
Trial registration number: Not applicable.

P-624 Investigating genetic associations with endocrine hormone levels in women undergoing fertility treatment

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Study question: Are single nucleotide polymorphisms (SNPs) associated with baseline serum hormone levels and endocrine signaling pathways in women?
Summary answer: We found that a SNP in the FSHR gene is associated with both AMH and day 2/3 FSH levels.
What is known already: The hypothalamic-pituitary-ovarian (HPO) axis and AMH hormone action are both important signaling pathways that promote oocyte growth and maturation. Disruptions to this signaling pathway can lead to subfertility, infertility, or other infertility indications. Research groups have shown that endocrine levels in women are affected by genetic influences. For example, SNPs within FSHR have been linked to FSH levels, which have been postulated to affect ovarian response in assisted reproductive technology (ART) treatments.
Study design, size, duration: We employed a retrospective cohort study design to identify significant associations between endocrine hormone levels and endocrine hormone SNPs. A total of 189 female study subjects were included in the study. Each participant consented to research in a de-identified manner. Patients were enrolled in the study between May 2014 and November 2015.
Participants/materials, setting, methods: The following exclusion criteria was used: PCOS diagnosis, FSH >11 mIU/mL, LH >30 mIU/mL, and progesterone >3 ng/mL. AMH levels and day 2/3 serum FSH, LH, and progesterone levels were collected by chart review. Genetic polymorphisms within FSHR, FSHB, and AMH were measured using Illumina's Infinium HD Genotyping assay. Welch's t-test was used to test 58 associations between hormone levels and SNPs. A p-value of $p < 0.05$ was considered significant.
Main results and the role of chance: Of the 58 comparisons between endocrine hormone levels and related SNPs, we observed two significant associations. First, we found that FSH levels were higher in individuals carrying the wildtype genotype than in individuals identified as heterozygous or homozygous mutant for the FSHR p.S680N SNP (p = 0.0396). This finding validates previous research demonstrating that FSH levels are impacted by the FSHR gene. Second, we found that AMH levels were lower in individuals carrying the wildtype or heterozygous genotype than in individuals identified as homozygous mutant for the FSHR p.S680N SNP (p = 0.040). To our knowledge, this association has not been shown previously. However, one study focused on a PCOS patient population (a group of participants excluded from this study) showed no association between AMH levels and FSHR SNPs. These findings reinforce the genetic influences on HPO signaling. The other comparisons between hormone levels and SNPs were not found to be significant.
Limitations, reasons for caution: A larger sample size may identify other significant associations this study did not observe. Additionally, the pool of study participants were not controlled for based on ethnicity or age, both of which may act as confounding factors.
Wider implications of the findings: HPO signaling is highly interconnected; multiple SNPs inherited together may influence signaling efficiency in more complex ways than previously thought. Larger studies designed to investigate multifactorial influences on endocrine signaling will lead to a deeper understanding of the clinical implications.
Trial registration number: NA.

P-625 Investigating X chromosome structure during mouse pre-implantation development

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Study question: The aim is to analyse the structural organisation of the X chromosome in pre-implantation embryos at the single cell level.
Summary answer: DNA FISH analysis of sub-megabase scale regions reveals major transitions in structural organisation of the mouse X between the 2 and 8-cell stages of development.
What is known already: Using Chromosome Conformation Capture and super-resolution microscopy approaches to examine the X-inactivation centre, our lab previously uncovered the existence of discrete topologically associating domains (TADs) each spanning several hundred kilobases, that display preferential interactions *in cis* (Nora et al., 2012). TADs are conserved across cell types, as well as between human and mouse, and appear to represent functional units that partition the genome. However, when and where TADs are established during development, or whether they are simply inherited from the gametes, remains unknown.
Study design, size, duration: Not applicable. Experiments have been performed in our lab in between May and November 2015.
Participants/materials, setting, methods: Wild type mouse female (C57BL/6J background) induced by hormonal treatment are used to produce preimplantation embryos (2 and 8-cell stage). These embryos were used for 3D DNA FISH using two-color probes spanning several hundred kilobases within a TAD or spanning two TADs, and imaged with super-resolution Structured Illumination Microscopy (SIM). Images were treated using ImageJ software homemade macro describing the Pearson correlation coefficient in both channels. Results were processed with Origin software for statistical analysis.
Main results and the role of chance: A locus encompassing two TADs on the X chromosome was found to display a similar structural organisation in 8-cell stage embryos as previously described in mESCs (9 embryos examined, 41 signals examined and $p = 1.9.10^{-9}$, two samples *t*-test). However, at an anterior developmental stage (2-cell), a very different organisation is observed (59 embryos examined, 214 signals examined and $p = 1.8 \times 10^{-3}$, two samples *t*-test). This suggests that TAD organisation may occur sometime between the 2- and 8-cell stages. This implies that genome reorganisation may occur following zygotic genome activation rather than being inherited from the gametes, although this requires further investigation. We are now examining different regions of the genome, on the X chromosome and autosomes.
Limitations, reasons for caution: Information obtained with 3D DNA FISH is limited to small regions of the genome, and too sparse for description of chromosomal organisation transitions. Chromosome Conformation Capture technique applied to single cells (Nagano et al; Nature 2013) is currently adapted in the lab to obtain chromosome-wide information for early mouse embryos.
Wider implications of the findings: It will be important to expand our findings to other mammalian species. Human embryos obtained through IVF procedure and given up for research will be used to repeat similar DNA FISH analyses.
Trial registration number: N/A.

P-626 Oocyte vitrification is a valid strategy to accumulate gametes for preimplantation genetic diagnosis for aneuploidy using next generation sequencing

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Study question: What is the relevance of aneuploidy in blastocyst from vitrified/warmed oocytes compared to fresh-oocytes from the same patients? How many surplus euploid embryos are obtained?
Summary answer: the percentage of aneuploidy in blastocyst produced from vitrified and fresh oocytes is similar. Oocyte vitrification increases the number of euploid viable blastocyst to transfer.
What is known already: Next Generation Sequencing is a valid technology for comprehensive chromosomal screening. Oocyte vitrification gives similar fertilization and pregnancy rates than fresh oocytes.
Study design, size, duration: The diagnosis of aneuploidies was performed from trophectoderm cells of blastocysts produced from fresh and vitrified/warmed oocytes on 51 infertile couples.
Participants/materials, setting, methods: 51 patients underwent a first ovarian stimulation to accumulate oocytes and a second to microinject with partner sperm together with the previously accumulated and warmed oocytes. Few trophectoderm cells were removed on day 5–6 blastocysts produced from the two sources of oocytes. Whole genome amplification was performed on few trophectoderm cells. Sample libraries were prepared Ion plus fragment library kit. Enriched barcoded-samples libraries were sequenced on next generation sequencer. Biopsied blastocyst were vitrified.
Main results and the role of chance: 101 blastocysts from fresh and 100 blastocysts from vitrified/warmed oocytes were produced. 100% of the samples were amplified after whole genome amplification. Chromosomal analyses were completed for all samples. 43 blastocyst from vitrified oocytes and 35 from fresh oocytes from sibling patients (42.6% versus 35.0%, $p > 0.05$) were euploid. Implantation rates of warmed and transfer of euploid blastocyst were similar from the two sources of oocytes (8/15 from vitrified oocytes and 18/31 from fresh oocytes). PGS results were confirmed after prenatal diagnosis.
Limitations, reasons for caution: The number (51) of infertile patients treated for PGS from vitrified and fresh oocytes remains limited. As described in the scientific literature, there can be discordance between chromosomal status of inner cell mass and biopsied trophectoderm cells. In NGS analysis, the percentage of aneuploidy should be measurable.
Wider implications of the findings: Preimplantation Genetic Diagnosis for aneuploidy on day 5 can lead to a limited number of euploid embryos to transfer or transfer cancellation. Oocyte vitrification and accumulation increases the chance to transfer an euploid blastocyst. This strategy can be applied to poor prognosis patients.
Trial registration number: no trial registration number.

P-627 Endometrial receptivity array (ERA) and the use of personalized embryo transfer (pET) in cases undergoing comprehensive chromosomal screening (CCS) for implantation failure (IF)

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Study question: This study aims to analyze the relative efficiency of endometrial receptivity assessment, personalized embryo transfer and CCS in improving the cycle outcome in IF cases.
Summary answer: The results show that pET can be positively associated to the cycle outcome, which can be maximized by inclusion of CCS in older patients.
What is known already: Recent studies indicate that, there exists an increased percentage of displacement regarding the endometrial window of implantation (WOI) in patients experiencing recurrent implantation failures. However, the relative contribution of WOI displacement with respect to the number of previous treatment failures, the age of the female as well as the corrective contribution of pET together with the availability of euploid blastocyst transfer in IF have not yet been assessed in the literature.
Study design, size, duration: This prospective cohort study was performed in Bahçeci Fulya IVF Centre and includes 132 frozen embryo transfer (ET) cycles of 195 patients whose WOI have been assessed by ERA between November 2013 – November 2015.
Participants/materials, setting, methods: All female patients included in the study were devoid of any known endometrium-related pathologies. An endometrial biopsy was taken from the uterus fundus by the help of a pipelle either on day LH+7 in a natural cycle or on day P+5 in an HRT cycle. The distribution of ERA results and the impact of pET strategy in our study population (number of previous trials, presence of advanced maternal age etc.) has been retrospectively analyzed.
Main results and the role of chance: Overall, in 28.7% of the cases, ERA test displayed non-receptive endometrium. Subgroup analysis according to previous number of trials, presence of previous early miscarriages as well as female age showed no significant differences ($p > 0.05$) among the groups, but a similar proportion of WOI displacement was stably present in all subgroups studied. Performing pET in these patients sufficiently ameliorated the pregnancy and implantation rates. In 69 cycles, a FET has been performed according to ERA results-only and in 63 cycles, frozen embryo replacements were performed in combination with ERA results and euploid blastocyst(s) after CCS. In frozen ET cycles where females were >38 years, the use of ERA and CCS together have been found to be maximize the clinical outcome as compared to ERA-only cycles ($p < 0.02$).

Limitations, reasons for caution: This study includes a large series of cases in which a possible contribution of endometrial receptivity assessment has been evaluated retrospectively. Well–designed ITT analyses and large prospective randomized studies are needed to better evaluate the most optimal use of such assessment approaches in the near future.
Wider implications of the findings: Our results can show that, in cases with IFs, application of ERA test and subsequent pET in younger females can improve the pregnancy rates by approximately 20%. In older females (≥38 years), pET can only be beneficial when combined by frozen ET using euploid embryos after CCS.
Trial registration number: None.

P-628 An ESHRE PGD Consortium multicentre retrospective cohort study investigating the clinical utility of PGD with human leukocyte antigen (HLA)-matching and factors influencing a positive outcome

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Study question: Has PGD-HLA been successful relative to diagnostic and clinical efficiency? Which parameters (assisted-reproduction (ART), embryology, genetic diagnosis) influence likelihood of donor-live-born baby/babies and transplantation?
Summary answer: Genetic probability remains a major limitation to overall success; further data analysis should highlight any influence from other variables in ART, embryology and genetic diagnosis.
What is known already: The first clinical cases for PGD-HLA were reported in 2001. It is now a well-established procedure, with an increasing number of cycles performed every year. However, PGD-HLA is still offered by relatively few PGD centers, including only 15% of the PGD Consortium members (based on annual data collections). The currently available data is fragmented and most reports on PGD-HLA application are limited in number and scope. Published systematic details on methodology, diagnostic results, overall ART success and haematopoietic stem cell transplantation (HSCT) outcomes are limited, precluding an evaluation of the true clinical utility of PGD-HLA cycles.
Study design, size, duration: Retrospective multi-centre cohort study aimed at investigating aspects of PGD-HLA cycles (performed 2001–2015) influencing positive outcomes: birth of genetically suitable donor-baby(ies) and HSCT. In April 2014, 30 PGD centres (Consortium members and non-members) with published/known PGD-HLA activity were invited to participate. Between February-September 2015, 14 centres submitted their data, through a custom-designed secure database, with login access (unique/centre). Data parameters covered all aspects of PGD-HLA cycles (ART, embryology and genetic diagnosis), donor-babies born and HSCT.
Participants/materials, setting, methods: From 716 cycles submitted by 14 centres (performed 2001–2015), data quality evaluation excluded 12 cycles, leaving 704, from 364 couples. The online database, based on Redcap, a free, secure, web-based data-capture application, was customized by CLEO (Collaborative Center for Clinical Epidemiology and Outcomes Research), Athens. Continuous variables are presented using mean, standard deviation, median and interquartile range, and categorical variables using absolute and relative frequencies. Data management and statistical analysis used STATA SE v.11.
Main results and the role of chance: Data included >700 HLA-PGD cycles performed between August 2001 and September 2015. Mean maternal age was 33 years. Most cases (81%) involved HLA-typing with concurrent exclusion of a single monogenic disease (58% with beta-thalassemia). In 92.5% couples, both partners were fertile, with average 1.93 HLA-PGD cycles/couple. Overall, 9751 oocytes were retrieved (13.89/cycle) and 5552 embryos analyzed (7.88/cycle). Most cycles involved fresh oocytes (94.87%) and day-3 embryo biopsy (85%). In 97% of cycles the genotyping method involved PCR only. 56.6% of couples achieved an embryo transfer (ET). Of 4392 embryos diagnosed (79% of analyzed), 644 were genetically suitable (16.2% of those analysed for HLA alone, ≈10% of those analysed for HLA with exclusion of monogenic disease). 598 embryos were transferred in 382 cycles, leading to 163 HCG-positive pregnancies (pregnancy rate/ET 42.67%, pregnancy rate/initiated cycle 24.3%). Until September 2015, 127 babies had been born, with 30 pregnancies ongoing. HSCT was performed in 55 cases (7.8% cycles initiated), of which 67% involved combined umbilical cord-blood and bone marrow transplantation from the HLA-identical sibling donor; 76.1% of transplants reported no complications. Technical aspects of HLA-PGD protocols, diagnostic findings, variable practice between centres, and other parameters potentially associated with a positive outcome will be discussed.
Limitations, reasons for caution: The findings of the study may be limited as not all PGD centres with PGD-HLA experience participated. Furthermore the study is based on retrospective data collection, from centres with variable practices and strategies for ART, embryology and genetic diagnosis.
Wider implications of the findings: This is the first multicentre study evaluating clinical utility of PGD-HLA, indicating variations in practice and outcomes throughout 15 years and between centres. Complete data analysis should highlight those parameters important for positive outcomes, approaches for further optimization, and provide health-practitioners and patients important information prior to initiating a cycle.
Trial registration number: Not a registered trial, but partly funded by ESHRE to customize the Redcap database and support data analysis by Drs. Georgia Kourlaba and Eleni Kourkouni, CLEO. The study also wishes to acknowledge Athens University Research Institute for the Prevention and Treatment of Genetic and Malignant Diseases of Childhood, Athens, Greece, for supporting PGD in the Department of Medical Genetics, University of Athens.

P-629 AMH and AMHR2 genes sequence variations in poor responders patients and controls – a pilot study

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Study question: Could genetic variations of *AMH* and *AMHR2* predispose infertile women to poor ovarian response?
Summary answer: Sequence variations in *AMH* gene and its signaling pathway (*AMHR2*) can influence AMH function and may be important to regulate ovarian reserve.
What is known already: The rate of follicular atresia is not equal for all women and genetic factors have been implicated in 5–15% of cases primary ovarian insufficiency. Ovarian reserve dictates the response to controlled ovarian stimulation and, to some extent, the pregnancy rate after IVF. Multiple studies have shown that variants of the *AMH* and *AMHR2* genes are associated with reproductive patterns of women, namely follicular phase E2 levels, unexplained infertility and age at natural menopause, suggesting a role of the AMH signaling pathway in the regulation of ovarian reserve.
Study design, size, duration: Cross-sectional pilot study where the entire coding sequence and the intron-exon junctions of the genes *AMH* and *AMHR2* were analysed in 34 infertile women with poor ovarian response. The variations found were then analysed in silico and then in 75 fertile (parous) controls.
Participants/materials, setting, methods: We recruited 34 infertile poor responder patients, as strictly defined by the Bologna criteria, and 75 controls at Centro Hospitalar e Universitário de Coimbra, Portugal (CHUC) and an

informed consent was obtained. The entire coding sequence and the intron-exon junctions of the genes *AMH* and *AMHR2* were analysed by Sanger sequencing in the study group and screened in the controls. An *in silico* analysis was performed using PyMol®.
Main results and the role of chance: A total of 20 different sequence variations were found: 14 in *AMH* and 6 in *AMHR2* gene, 3 of which were novel, further submitted to *in silico* analysis. This analysis reveals that 4 heterozygous alterations in *AMH* gene have a predictable functional impact. A preliminary screening showed that the alterations p.Asp288Gln and p.Gln185Glu are absent in 75 controls. Prediction by PyMol® analysis reveals that the mutated proteins have a significantly different structure when compared with the normal protein.
Limitations, reasons for caution: Reduced sample size, since it is a genetic study limited to a Portuguese population sample.
Wider implications of the findings: This study reveals new insights into the pathogenesis of ovarian insufficiency. There are no similar studies performed in European patients and our results agree with previous studies showing that variants of the *AMH* and *AMHR2* genes are associated with reproductive patterns, unexplained infertility and age at natural menopause.
Trial registration number: 041-CE-2013.

P-630 Validation of the de-novo segmental (>16 megabase) loss detected by next generation sequencing in 24 blastocysts from preimplantation genetics diagnosis

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Study question: Whether the de-novo segmental abnormality above 16 megabase (Mb) detected in next generation sequencing–preimplantation genetics diagnosis (NGS-PGD) is authentic?
Summary answer: De novo segmental abnormalities above 16 Mb occurred in blastocysts and could be detected by NGS, but some existed as mosaicism in TE and ICM.
What is known already: Recently, NGS become a routine technique to be applied in PGD that can screen the whole chromosomal set and detect de-novo segmental abnormality. De novo chromosomal abnormalities had been validated to be accurate in many publications but the anthenticity of the de-novo segmental abnormalities, especially for those abnormalities above 16 Mb that usually would be reported have not been validated.
Study design, size, duration: The study was set in an IVF center at the Reproductive and Genetic Hospital of CITIC-Xiangya. Retrospective studied of 1759 embryos from Robertsonian or reciprocal translocation or inversion carriers that analysed by NGS-PGD between April 2013 and December 2015. Meanwhile using FISH to validate the de-novo segmental loss (>16 Mb) in 24 non-transferred blastocysts donated for research.
Participants/materials, setting, methods: Twenty-four blastocysts with the de-novo segmental loss (range from 16 Mb to 147.7 Mb) were thawed, all the blastocyst were then cut into half with either trophectoderm (TE) part and inner cell mass (ICM) part. All materials were separately fixed and analyzed by FISH using a combination of probes (locus-specific identifiers, centromeric and subtelomeric), specially designed to properly validate the de-novo segmental abnormalities. The FISH results were compared with those of NGS-PGD.
Main results and the role of chance: The frequency of the de-novo segmental abnormalities in embryos which applied NGS-PGD were 13.5% (238/1759), and there were 98 embryos exhibited only the de-novo segmental abnormalities. The de-novo segmental gain or loss discovered by NGS were occurred on all chromosomes, mainly on the chromosome 3, 8, 11, 12, 18 and X. For the 24 blastocysts, 23 (95.8%) blastocysts were successfully thawed and reliable FISH results were obtained; the remaining one had no result because of the degradation after thawing. After FISH analysed, 20 (86.9%) of 23 de-novo segmental loss detected by NGS were confirmed. The FISH analysis did not detect the de-novo segmental loss in three blastocysts, in which all cells analyzed were normal. Among the 20 blastocysts, 9 blastocysts had the de-novo segmental abnormalities in both TE and ICM, 11 blastocysts are mosaic with both de-novo segmental loss and normal signals. However, the mosaicism were only detected in the TE, and the corresponding ICM had normal signals in 2 blastocysts.
Limitations, reasons for caution: The sample size in the validation study is small and could not had consolidate conclusion on the results observed above. Further study with a larger sample size and a set up for both gain and loss may be helpful.
Wider implications of the findings: The preliminary study indicated that de-novo segmental abnormalities exist in human blastocyst from in vitro fertilization and may account partially for the implantation failure.
Trial registration number: Not application.

P-631 The proportion of aneuploidies is associated with the clinical outcome in patients with repetitive implantation failure undergoing pre-implantation genetic screening by aCGH

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Study question: Repetitive implantation failures (RIF) may have different origins: is pre-implantation genetic screening (PGS) able to identify genetic cause from other causes?
Summary answer: PGS is able to identify the main cause of previous failures (genetic or other factors) when considering the proportion of aneuploidies.
What is known already: The repetitive implantation failure of good-quality embryos can have multiple causes but no diagnostic tests are available to identify the etiology. Therefore, the therapeutic strategies proposed can produce controversial results and are not able to identify the etiology. Oocytes/embryos defects are considered one of the potential cause of RIF and PGS is offered to these patients since years. Data from retrospective and prospective studies shows that, overall, RIF patients have more chromosomal abnormalities compared to control groups. However, the usefulness of PGS in terms of clinical outcome is still controversial, since other factors can be involved.
Study design, size, duration: Retrospective analysis of 60 RIF couples (failure of ≥3 transfers with good-quality embryos, female age ≤ 40 yrs, normal ovarian reserve, normal karyotype, normal uterine cavity) undergoing PGS by aCGH between April 2012 and April 2014. A severe male factor was present in 13 couples. The control group consists in a cohort of 77 RIF couples having the same criteria but not undergoing PGS.
Participants/materials, setting, methods: According to the policy adopted during the study period, couples without a severe male factor underwent polar bodies biopsies and aCGH analysis if zygote developed normally to 6–8 cells (47 couples, group A); couples with a severe male factor underwent PGS on blastomere (13 couples, group B). For the analysis of outcomes (LBR/patient), group A was divided depending on the proportion of aneuploidies: <50% and >50%.
Main results and the role of chance: No differences resulted between control and PGS cycles in the number of eggs (8.1 ± 4.5 vs 8.7 ± .4.0) and in the fertilization rate (78%vs 76%). The mean female age was 37.5 in PGS and 36.4 in control. In controls, the LBR was 26% Overall, in the PGS group, the incidence of aneuploidies was 69% (205/295) and the LBR was 20%. In group A PGS cycles (no severe male factor): 25 patients (53%) had a proportion of aneuploidies <50%. This group had a high probability to be transferred but the LBR (20%) was not improved by the transfer of embryos selected by PGS; other factors may be involved. 22 patients (47%) had a proportion of aneuploidies >50%. Those patients had a high risk to be not transferred (9/13), but when at least one embryos was available for transfer, the LBR (46%) resulted improved by PGS, suggesting that the main cause of the previous failure was a genetic factor. In group B PGS, the incidence of aneuploidies was 75%, 5 patients were transferred but even the transfer of euploid embryos was not able to improve the outcome (LBR 20%) suggesting complex abnormalities in embryos.
Limitations, reasons for caution: The study is a retrospective analysis and the number of cases is limited. However, the published studies on RIF and PGS do not usually include a significant higher number of cases and no studies analyzed the data depending on the proportion of aneuploidies.

Wider implications of the findings: RIF is a big challenge since its multiple causes and because no diagnostic tests are available to identify the main factor. Therapies are empirical and produce controversial results. Our preliminary data suggest that the proportion of aneuploidies by PGS can have a diagnostic role in identifying the RIF etiology.
Trial registration number: Not applicable.

P-632 A novel preimplantation genetic diagnosis strategy for Duchenne muscular dystrophy based on targeted next-generation sequencing and linkage analysis

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Study question: To establish a rapid and broadly applicable strategy of PGD for DMD carriers based on target sequencing and linkage analysis.
Summary answer: Targeted next-generation sequencing (NGS) combined with SNP linkage analysis is a efficient strategy than previous methods and being applicable to 99% of DMD carriers.
What is known already: DMD is the most common fatal genetic disorder diagnosed in childhood, with an incidence of 1/3500 in live male births. The gene is about 2.3 Mb and has 79 exons. Multiple mutations were found, including large deletions (60%), large duplications (10%) and point mutations (30%). The traditional PGD approaches take up to three months to complete the pre-experiment. Although karyomapping is a faster PGD technology and suit for a wide range of conditions, it is not suit for DMD due to inadequate SNPs. Now we are still lack of a high efficiency and easy to clinical extension approach for DMD-PGD.
Study design, size, duration: Targeted NGS workflow based on an AmpliSeq panel was designed for sequencing 480 SNPs and coding region of DMD gene on Ion PGM™ Sequencer. Total 99 female carriers with DMD heterogeneous mutation were recruited for testing the application range. Of them 3 patients were carried out 3 PGD cycles to verify the efficacy of the novel DMD-PGD scheme. The work was carried out from January 2014 to December 2015.
Participants/materials, setting, methods: The study was set at the Reproductive and Genetic Hospital of CITIC-Xiangya, China. The designed AmpliSeq panel includes the coding region of DMD gene and 480 SNPs selected from 1 Mb upstream to 1 Mb downstream of the gene. Ninety-nine females carrying heterogeneous DMD gene mutation, including 51 large deletions, 15 large duplications and 32 point mutations, were recruited to determine the efficacy of the panel. Among them 3 carriers underwent PGD and 14 embryos were tested.
Main results and the role of chance: Among the 99 female DMD carriers, 98 had at least 3 informative SNPs in the upstream and downstream of the mutation loci of the gene, respectively. The successful rate of haplotype construction was 99% (98/99). The point mutations were detected in all 32 carriers and the efficacy rate was 100%. Of the 14 embryos from 3 PGD cycles for 3 families, 1 was normal male embryo, 4 were normal female embryos, 5 were female carrier embryos and 4 were in affected embryos. Among them, 2 families were transferred 2 normal female embryos and 1 female carrier embryos, respectively. The PGD results were confirmed by the following prenatal diagnosis.
Limitations, reasons for caution: This strategy can not directly detect the large deletions and duplications of DMD gene. In addition, 1 case (1%) had less than 3 informative SNPs within 1 Mb upstream and 1 Mb downstream of the mutation loci of the gene, which might decrease the accuracy of the results.
Wider implications of the findings: We establish a new DMD-PGD strategy based on targeted NGS and linkage analysis. Ninety–nine percent of female carriers were successfully constructed haplotype. The new scheme is rapid, accurate, low cost and easy to clinic extension.
Trial registration number: Not applicable.

P-633 Next generation sequencing (NGS) metrics following DOP-PCR whole genome amplification (WGA) of single and multi-cell samples for PGS

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Study question: Evaluate single cell and limited DNA template sample NGS metrics following WGA and sequencing on alternate capacity sequencers.
Summary answer: The amount of sequencing data available for interrogation is highly dependent on the number of samples multiplexed in a library, read length and platform configuration.
What is known already: When limited source material for genome wide evaluation is available, WGA is often used to generate sufficient DNA for downstream analysis. Limited WGA technologies are commercially available, and WGA using the EmbryoCellect™ kit (Reproductive Health Science Ltd) protocol utilizes a proprietary degenerate oligonucleotide primed PCR (RHS-DOP-PCR). When used in conjunction with the kit array, screens low template samples for aneuploid status. Aneuploidy screening using NGS technology is becoming increasingly popular in the IVF setting. The total aligned genomic and mitochondrial DNA sequence data generated from an NGS run is potentially sufficient for subsequent sample screening for other genetic anomalies.
Study design, size, duration: The aim of this study was to determine NGS run and aligned read data metrics from a range of NGS platforms as models for PGS utilizing the DOP-PCR based WGA of single cell and multi-cell aliquots as described (EmbryoCellect™ kit). Additionally, fluorescently labelled test and reference WGA DNA was hybridized to the EmbryoCellect™ kit microarray, consisting of repeat-depleted chromosome-specific probes. The dye ratio was determined and compared to the known karyotype of the sample.
Participants/materials, setting, methods: Single cell and 5-cell aliquots sorted from euploid and aneuploid cell lines (Coriell Institute) were subjected to WGA using EmbryoCellect™ kit protocol. Nextera libraries were prepared from WGA and unamplified gDNA samples and subsequently sequenced (paired-end) on either a MiSeq (n = 48; read length 300 bp), NextSeq (n = 23; 150 bp) or X-10 (150 bp) platform according to standard protocol (Illumina). The sequencing data was bioinformatically aligned to hg19. Sequencing run and aligned data metrics were tabulated for comparison.
Main results and the role of chance: On completion of the sequencing runs, approximately 4.5 fold more reads were generated using the NextSeq compared to MiSeq platform (mean; 1.3 million reads versus 5.8 million reads per sample were mapped to hg19). The sequencing of single cells and 5-cell aliquots on the X-10 yielded 110,000–120,000 Mb in comparison to 126 000 Mb from sequencing of the unamplified gDNA sample or approximately 400 million reads per sample. The average single cell Q30 scores were >89% for the Miseq run and >82% for the NextSeq NGS run. The average X-10 Q30 scores for single cells, 5-cell aliquots and gDNA were 83%, 81% and 87% respectively.
Limitations, reasons for caution: This data has been generated from limited NGS runs, and therefore increasing the number of sequencing runs is necessary to increase the number of samples per sequencing parameter/condition.
Wider implications of the findings: While specific gene sequence and mitochondrial DNA is present in single cell NGS data, breadth and depth of coverage to acquire a level of resolution suitable for PGD will be a key indicator for PGS + PGD success.
Trial registration number: –.

P-634 Development of a universal method for the preimplantation diagnosis of β-thalassemia and sickle-cell anaemia using a novel next-generation sequencing approach: a new paradigm for PGD

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Study question: To develop a novel, low-cost PGD protocol applicable to all couples undergoing PGD for β-thalassemia and sickle-cell anaemia.
Summary answer: The study successfully harnessed the power of next-generation sequencing (NGS) technology, delivering a PGD protocol for diagnosis of any *HBB* gene mutations in preimplantation embryos.
What is known already: Worldwide, the most common inherited disorders are those caused by mutation of the beta-globin gene (*HBB*), responsible for beta-thalassemia and sickle cell anaemia. There are various challenges in providing

PGD for these conditions. Traditional PGD protocols involve the time-consuming process of designing customized tests for each mutation. This is problematic in the case of *HBB* as there is a wide diversity of mutations. Not only does the need to develop customized tests substantially delay treatment, but the work entailed greatly increases the cost of PGD. This is problematic considering the high prevalence of *HBB* mutations in many resource poor countries.

Study design, size, duration: A large multiplex PCR protocol was designed, allowing simultaneous amplification of multiple overlapping DNA fragments encompassing the entire *HBB* gene sequence. Additionally, 22 linked polymorphisms (SNPs) flanking the *HBB* gene were amplified in additional PCR fragments. The resulting DNA was subjected to NGS to reveal the genotype/mutation status.

Participants/materials, setting, methods: The protocol was validated in samples from 4 families carrying different β-thalassemia mutations. The method was also applied to whole-genome amplified (WGA) DNA from 24 embryos derived from couples carrying various mutations in the *HBB* gene (thalassemia and sickle cell). In total, 12 mutations were assessed and the results obtained using the new methodology were compared to those obtained from conventional PGD methods for diagnosis of single gene disorders.

Main results and the role of chance: The new NGS-based protocol accurately detected the mutations in the DNA samples tested, confirming all patient genotypes. Seventeen single nucleotide polymorphisms (SNPs) in close proximity to the *HBB* locus (all within 1 Mb) were also successfully sequenced. Five further SNPs were identified within the *HBB* gene locus (i.e., intragenic) in the patient samples. Of the 22 SNPs analysed, each was informative for at least one family. On average, the families tested had 10 informative SNP loci. This allowed the inheritance of haplotypes associated with mutant genes to be tracked with high precision, providing a supplementary means of diagnosis, additional to direct mutation detection. The combination of direct mutation detection and analysis of multiple informative polymorphisms provides a redundant diagnostic, highly resistant to misdiagnoses due to problems such as allele dropout. Concerning the WGA DNA derived from embryo biopsy specimens, the protocol was able to correctly diagnose all 24 associated embryos. When compared to the results obtained from conventional PGD or karyomapping the new test displayed 100% concordance. Importantly, no patient-specific test design or optimization was needed. As far as we are aware, this is the first report of an NGS-based method for PGD of a monogenic disorder.

Limitations, reasons for caution: As part of the clinical validation of this newly-designed protocol for diagnosis of β-thalassemia and sickle-cell anaemia in preimplantation embryos, analysis on additional embryos with a wider variety of mutations should be undertaken. This will permit a more robust and comprehensive evaluation of the sensitivity and specificity of the method.

Wider implications of the findings: For disorders characterized by large numbers of different mutations, NGS-based PGD protocols, such as that described here, provide a simple generic approach, which is substantially less time-consuming and more cost-effective than conventional PGD methods. Lower costs should improve patient access to PGD, especially in less affluent parts of the world.

Trial registration number: N/A.

P-635 Clinical outcome of SET transfer of euploid embryos by Next Generation Sequencing with and without MitoGrade (mitochondrial DNA selection)

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Study question: What is the overall value of mitochondrial DNA (mtDNA) assessment in a clinical setting, and how can it help improve IVF outcomes?

Summary answer: Retrospective analysis demonstrates that mtDNA quantification has a high negative predictive value and can be used as an independent biomarker in determining embryo viability.

What is known already: Chromosomal abnormalities are a major cause of implantation failure. Recently our group has validated the relationship between mtDNA quantification and IVF outcomes in a non-selection study. In particular we have been able to establish a threshold above which the probability of implantation is greatly reduced. While further randomized testing is being conducted, we took this opportunity to retrospectively analyze the overall value of the established mtDNA threshold in previously completed clinical cases.

Study design, size, duration: Retrospective study in which mtDNA was assessed in a total of 572 euploid blastocysts obtained from 328 couples (average maternal age 34.95 ± 0.27 years) undergoing preimplantation genetic screening (PGS). Outcome data was collected from 6 different IVF centers for routine follow up. Implantation outcomes were then utilized to determine the validity of the established threshold.

Participants/materials, setting, methods: DNA from blastocyst biopsy samples was amplified (Sureplex, Illumina, USA) and then subjected to aneuploidy analysis using next generation sequencing (NGS, Veriseq protocol, Illumina, USA). Only those embryos classified as chromosomally normal had their mtDNA levels assessed using MitoGrade (Reprogenetics). mtDNA levels were then compared to the pregnancy outcomes to confirm implantation predictions. All embryos were single embryo transfers (SET).

Main results and the role of chance: Nearly 14% (80/572) of all blastocysts analyzed contained mtDNA levels above the established threshold and were predicted to have lower chances of implantation. To date, 246 euploid embryos were replaced in SET with a pregnancy rate of 62.3% (153/246). Retrospective assessment of mtDNA levels revealed 216 embryos to contain normal mtDNA levels. Therefore the pregnancy rate post mtDNA quantification was 71% (153/216) in the normal level mtDNA group. Furthermore of the 30 embryos with elevated mtDNA levels, only one led to a successful pregnancy. Therefore the negative predictive value of mtDNA quantification was 96.7% (29/30). This highly significant ($p < 0.001$) difference between implanting and non-implanting embryos validates the clinical applicability of mtDNA quantification.

More importantly, the mtDNA threshold retained its validity across six different IVF centers and was unaffected by maternal age.

Limitations, reasons for caution: The study was retrospective and the number of cycles was not enough to show a difference between no selection (62% pregnancy rate) and selection against MitoGrade elevated embryos (71% pregnancy rate). It was however large enough to show a significant difference in implantation rates between elevated and normal MitoGrade embryos.

Wider implications of the findings: This study demonstrates the validity of MitoGrade as an independent variable in predicting embryonic implantation potential of euploid embryos. Further research involving the biological significance of mtDNA levels and implantation rates would be invaluable.

Trial registration number: N/A.

P-636 XRCC1 polymorphism Arg399Gln is associated with male infertility

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Study question: Is XRCC1 polymorphism Arg399Gln associated with male infertility?

Summary answer: The Gln variant of the XRCC1 polymorphism Arg399Gln is associated with a decrease in the risk of male infertility.

What is known already: XRCC1 is a critical enzyme in the BER DNA repair system. Its expression in testis in higher than in any other tissue; it appears to have an important role in repairing DNA damages during spermatogenesis. XRCC1 polymorphism Arg399Gln is located in the PARP (poly-ADP ribose polymerase) binding domain.

Study design, size, duration: Case-control study with 120 seminal samples from infertile patients and 79 from donors with probed fertility.

Participants/materials, setting, methods: All samples where genotyped using the PCR-RFLP system. The primers used in the PCR where F 5′-TTGTGCTTTCTCTGTGTCCA-3′ and R 5-TCCTCCAGCCTTTTCTGATA-3′ and the selected restriction enzyme was the MspI.

Main results and the role of chance: The allele frequencies where 0.66 (Arg) and 0.34 (Gln) in the patients group and 0.56 (Arg) and 0.44 (Gln) in the controls group. The genotype frequencies were 0.488 (Arg/Arg), 0.344 (Arg/Gln) and 0.172 (Gln/Gln) in the patients group and 0.278 (Arg/Arg), 0.557 (Arg/

Gln) and 0.165 (Gln/Gln) in the donors group. We observed significant differences in the allelic frequencies distributions between patients and donors (p = 0.006). We also observed that the patients group allele frequencies did not adjust to a Hardy-Weinberg equilibrium (p = 0.0087), appearing less heterozygous than expected. We hypothesize that the Gln variant may be a more active enzyme.
Limitations, reasons for caution: Our population only includes Spanish population.
Wider implications of the findings: There is no consensus among the literature regarding the effect of this particular polymorphism over fertility. Our findings may help to clarify this situation.
Trial registration number: Not a clinical trial.

P-637 Clinical application of sequencing-based preimplantation genetic diagnosis

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Study question: current techniques are restricted to a few specific diseases and preliminary experiments in the lab always take long time to confirm the linkage SNP or STR for each family case.
Summary answer: We believe the sequencing-based preimplantation genetic diagnosis would served as a more effective, useful and routine techniques in the lab-level medical centers.
What is known already: Since the first case of sex determination in embryos with sex-linked diseases in 1990s, advanced technologies for preimplantation genetic diagnosis (PGD), such as PCR-based molecular techniques, Sanger sequencing, short tandem repeats (STR) linkage analysis and SNP mapping, has been quickly developed and optimized for accurate detection for monogenetic diseases, for example, α and β thalassemia.
Study design, size, duration: 34 monogenetic diseases with relatively high prevalence was involved in this study, including autosomal recessive/dominant and X-linked diseases. Families who have clear medical history and want to have a healthy baby through preimplantation genetic diagnosis in a IVF center, were reviewed and recruited for the study.
Participants/materials, setting, methods: Here we recruited seven families affected by monogenetic diseases from April to November 2015 in our hospitals, including one case of Hemophagocyic Histiocytosis, one case of Osteogenesis imperfect, three cases of polycystic kidney, one case of Spinal Muscular Atrophy and one case of Wilson's Disease. We combine the quick detection of causative mutations in 34 monogenetic diseases at one time for the victims and PGD for embryos with a haplotype-based approach.
Main results and the role of chance: We combine the quick detection of causative mutations in 34 monogenetic diseases at one time for the victims and PGD for embryos with a haplotype-based approach. we successfully phased the local haplotype across the interest gene or chromosome for each embryo and helped to select healthy embryos for implantation. The PGD results were concordant with prenatal diagnosis by conventional techniques in amniotic fluids, showing 100% accuracy. We believe the sequencing-based preimplantation genetic diagnosis would be faster and cheaper in the near future and served as a more effective, useful and routine techniques in the lab-level medical centers.
Limitations, reasons for caution: We believe more comprehensive evaluation across different hospitals as well as more monogenetic diseases should be performed in the future, to get a better understanding the technology, a lower cost in practice, a faster turnaround time and easier accessible training system for general clinicians in this field.
Wider implications of the findings: We believe the sequencing-based preimplantation genetic diagnosis faster and cheaper and served as a more effective, useful and routine techniques in the lab-level medical centers in the near future.
Trial registration number: No.

P-638 Relationship between mitochondrial DNA quantity assessed by next generation sequencing (NGS) in blastocyst stage embryos and their implantation

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Study question: The aim of our work was to indirectly estimate if mitochondrial DNA relative amount from 5th day biopsy procedure differs between embryos implanted and those that failed to implant.
Summary answer: Implanted embryos exhibited significantly higher amounts of mitochondrial DNA indicating mtDNA as a possible new biomarker for embryo prioritisation.
What is known already: Application of the preimplantation genetic screening (PGS) in reproductive medicine seems to be one of the most important improvement in treatment of human reproductive problems. Considering the current state of technological progress and available already perfected, validated methods, next generation sequencing (NGS) appears to be the most flexible. Among the available methods Next generation sequencing gives us additional diagnostic possibilities enabling us to obtain data from sequencing of mitochondrial DNA (mtDNA) while testing genomic DNA.
Study design, size, duration: One hundred and four patients treated with intracytoplasmic sperm injection (ICSI) at our IVF centre between August 2013 and March 2015 who decided to undergo PGD on 24 chromosomes on blastocysts with frozen embryo transfer were included in the investigation. Retrospective analysis was finally performed on 281 trophectoderm samples obtained from 5 day embryos.
Participants/materials, setting, methods: Trophectoderm cells were biopsied from embryos at the blastocyst stage and subjected to preimplantation genetic screening (PGS) by next generation sequencing (NGS). Obtained information regarding mtDNA copy number amount was compared between euploid and aneuploid as well between implanted and non-implanted. Successful implantation was defined by presence of gestational sac at 5 weeks and 3 days ±2 days after embryo transfer by transvaginal ultrasound.
Main results and the role of chance: Relative amount of mtDNA was lower in euploid than in aneuploid embryos [0.91 (0.6–1.7) 1.1 (0.7–2.1) p = 0.05] what is consistent with previous reports. Surprisingly, significantly higher rate of mtDNA was found for the implanted embryos [1.53 (1–2.2) vs 0.72 (0.5–1.3) in non-implanted, p = 0.005]. This greater mtDNA amount in implanted embryos may indicate a higher energetical potential and therefore likely better adaptive capabilities of the implanted embryos.
Limitations, reasons for caution: Our research does not categorically confirm the ratios of mtDNA to gDNA due to the whole genome amplification (WGA) procedure performed prior to NGS. WGA may result in altered proportions of readings of gDNA and mtDNA.
Wider implications of the findings: If these findings are confirmed by large multi-center studies, validated and repeatable results would enable introduction of the above described results into clinical practice.
Trial registration number: Not applicable.

P-639 Identifying housekeeping genes that are stably expressed in the endometrium of both fertile women and those with recurrent implantation failure and recurrent miscarriages

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Study question: Are there a set of House Keeping Genes (HKGs) that are stably expressed in the endometrium of fertile women and those with recurrent implantation failure and recurrent miscarriages?
Summary answer: Previously untested genes are more stable than those identified in the literature. We recommend three new HKGs for gene normalisation in endometrial tissues.
What is known already: The most commonly used HKG have been shown to vary across samples/tissues (Andersen et al., 2004; Vandesompele et al., 2002). Despite this, validation of the presumed stability of HKG in tissues such as the

endometrium is often not made (Colette et al., 2009; Matsuzaki, et al., 2006). There is no consensus on which HKGs are stably expressed in endometrial tissue but they vary with different pathologies (Sadek et al., 2012). This current failure may be because often the most commonly cited and used HKG are applied to disease states and pathologies in which they have not be tested nor verified.

Study design, size, duration: A case control gene expression study (August 2012 to December 2013, *n* =45) was performed in order to identify HKG that express inherent levels of stability across different patient endometrial tissues. Using data from >30,000 microarray experiments, pair-wise variation with the sequential addition of each HKG indicated the genes that could be used, with the normalisation factor as the geometric mean of the most stable gene.

Participants/materials, setting, methods: Women between 25–45 years were recruited and baseline demographics/fertility characteristics were collected. Women were included if they suffered recurrent miscarriage (RM) or recurrent implantation failure (RIF) or were fertile controls. Endometrial biopsies were taken by suction curette and total RNA extraction, cDNA synthesis and PCR was performed on all samples using a total of 18 HKG. The genes were arranged in terms of stability and normalisation was determined.

Main results and the role of chance: The present study using 18 HKGs found that genes not previously tested in endometrial samples are more stable than those previously identified as most stable in our patient groups. To establish the number of reference genes used to make this geometric mean, the 'V score' was utilised (this indicates how the normalisation factor changes when another gene is included in its calculation; and a 'V score' of 0.15 or below indicated that the additional gene had no significant contribution to the newly calculated normalisation factor). We recommend using PRDM4 (PR domain 4), UBE2D2 (Ubiquitin-Conjugating Enzyme E2D 2) and ENOX2 (Ecto-NOX Disulfide-Thiol Exchanger 2) as reference HKG for normalisation of endometrial tissues taken from patients with RM and RIF, as these appear to be the most stably expressed HKG regardless of endometrial pathology. While the genes we identified are applicable to studies of apparently normal endometrium, the investigation of a wider range of reference HKG may be more appropriate in order to identify the best candidates for studies of a given endometrial pathology.

Limitations, reasons for caution: Our results may be confounded by a small sample size; a larger scale study in this specific subgroup of women is further needed to confirm our findings.

Wider implications of the findings: The commonly used reference HKGs are not as stable as previously reported and the expression of HKG within the endometrium is affected by reproductive pathology. This information should be applied when using normalisation genes. Cellular functions of commonly used HKGs may have a role in these conditions.

Trial registration number: Regional ethics committee number 12/SC/0568.

P-640 A powerful tagging single nucleotide polymorphism method in terms of next-generation sequencing that combines preimplantation genetic diagnosis, informativity testing and aneuploidy screening

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Study question: Is our new NGS strategy that identifies point mutations by confirmatory tagging-SNP method reliable to determine mutation-free embryos avoiding the allele-drop-out (ADO) in PGD cycles?

Summary answer: We've developed and implemented a high-throughput tag-SNP genotyping combined with Ion-AmpliSeq™ sequencing (Thermo-Fisher Scientific) for the PGD of point mutations to ensure a sufficient informativity.

What is known already: PGD represents a reproductive option for couples at genetic risk of having a child with a specific monogenic disorder. PGD refers to genetic profiling of embryos prior to implantation and involves the use of assisted reproductive technology combined with in vitro fertilization (IVF) techniques. Only those disease-free embryos are transferred to the uterus with the aim of generating healthy offspring. Nowadays, PGD coupled with informativity testing on Short-Tandem Repeats (STRs) markers become mandatory before IVF. Analysis of SNPs linked to gene regions involved by mutation with NGS technology enables an all-in-one informativity testing, PGS and PGD shortly after embryo biopsy.

Study design, size, duration: This study comprises seven couples performing IVF cycles with PGS/PGD (November 2015–January 2016), where one or both members carried a point mutation. Two females were diagnosed as carriers of PKD1 gene mutations (c.1261C>T; c.1810C>T). One female carried one RET mutation: IVS9 (-1)bis(-2)delAG. One female carried mutation c.670delC on L1CAM. A couple carried two LAMB3 mutations (c.1903C>T in the female and c.810delC in the male). Other couple carried two UNC13D mutations (female: c.2346-2349del; male: c.1389+1G>A)

Participants/materials, setting, methods: Parental blood samples were required for informativity testing. LD Tag-SNP tool (NIEHS, US) provided linkage disequilibrium for SNPs selected within each gene. This tool prepared DNA sequences for primer design considering SNP information in 200,000 bp flanking region on both sides. Targeted next generation sequencing was performed using Ion-AmpliSeq™ 200 bp-primer pools and Ion PGM™ platform**.** After oocyte insemination and day-5 biopsy, DNA whole genome amplification was followed by PGS/PGD/informativity testing in all embryos.

Main results and the role of chance: A panel of around 130 different SNP markers has been studied during this set-up phase, to ensure a sufficient informativity in all cases. Database for all SNPs with European population genotype data was found in dbSNP. Around 15% of tag-SNP studied was fully informative in order to identify linked-point mutations carried by partners of couples studied. Along the PGS/PGD processes performed to date, no amplification failures in genomic regions involved by mutations, neither false positives nor negatives were detected. The overall ADO rate was no greater than 3%. The risk of double recombination between informative flanking markers was estimated in less than 0.05%. Regarding to one of the couples in which the female was diagnosed as PKD1 mutation carrier: c.1261C>T p.(Arg421Cys), 12 embryos resulted after one IVF cycle. NGS Ion PGM™ System along with Ion-ReproSeq™ workflow (Thermo-Fisher Scientific) determined that 4 were euploid, 4 aneuploid and 4 presented a chaotic arrangement. PGD/tag-SNP informativity testing in euploid embryos showed 2 mutation-free ones and 2 that presented the mutation in heterozygosis. Similar results were reported for the others cases. To sum up, the sample-tagging plan based on this framework will improve whole PGD/PGS in terms of turnaround time, reliability and cost-effectiveness.

Limitations, reasons for caution: Number of case-control studies is limited to the carriers of monogenic diseases. Higher throughput would be accomplished by multiplexing tag-SNPs genotyped over multiple candidate genes. Improving this power-based algorithm would be useful for an optimal selection of informative SNPs based upon maximizing the predictability of unmeasured SNPs or SNP haplotypes.

Wider implications of the findings: Conventional multiplex markers in polymerase chain reaction (PCR) protocols for PGD are laborious, expensive and requires large amount of DNA template. However, the application of tag-SNPs combined with Ion-AmpliSeq™ technology can be useful in capturing untyped SNPs information in a genomic region involved by mutation and improve PGD success.

Trial registration number: This is not a clinical trial so it not linked to a trial registration number.

P-641 Non invasive prenatal testing (NIPT) in IVF patients

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Study question: Is the non invasive prenatal testing (NIPT) a must to suggest to IVF population patients?

Summary answer: In a female population where invasive testing happen to increase the risk of miscarriage, we want to counsel patients for a non invasive alternative.

What is known already: Different companies developed NIPT to detect aneuploidies that are normally compatible with a live birth. Two main technologies

rule in these tests: the counting method and the single nucleotide polymorphisms (SNPs). This test is progressively become a first line choice during prenatal screening.
Study design, size, duration: This is a cohort study started on December 1st 2014 till November 30th 2015. Three thousand and seventy none couples performed a SNPs based NIPT and we analysed results and follow up. The test include either a basic panel with detection of trisomy 13, 18, 21 and sexual chromosomes X and Y or an expanded panel (NIPT+) that includes 5 microdeletions as well. Patients decided after counselling to undergo one or the other.
Participants/materials, setting, methods: In our centers, we counsel pregnant patients for:

- non invasive tests such as combined test and NIPT;
- invasive tests such as or chorionic villus sampling (CVS) and amniocentesis.

The NIPT is available after 9 + 0 weeks of gestation. The nurse withdraw two blood tubes from each woman and turn them upside down for 10 times. We keep the blood at room temperature. The cut off necessary to detect the fetal DNA signal is 4%.
Main results and the role of chance: Three thousand and seventy nine couples were tested, of that 1643 with the standard NIPT and 1436 used the panel with 5 microdeletion (NIPT+). The rate of no informative result due to low fetal fraction was 3.1% (51/1643) in the NIPT group and 4.2% (60/1436) in the NIPT+ arm. The number of high risk result was: 1.3% (20/1592) in NIPT and 2.3% (31/1376) in the NIPT+ group. Overall, we found 21 high risk for trisomy 21, 4 for trisomy 18, 6 for trisomy 13, 3 monosomy X, 1 triple X (XXX), 1 XXY, 4 XYY. As for the microdeletions we found 4 positive to Di George syndrome, 1 for p36, 3 positive for Angelman syndrome and 1 for cri du chat. When we obtain a positive result, the doctor always suggested an invasive follow up but a rate of patients did not accept it. Through CVS/amniocentesis 18 positive results were confirmed while 12 were false positive and not confirmed with invasive analyses. The remaining are either ongoing and did not accept invasive testing or lost of follow up. The microdeletion were confirmed in 1 case for a Di George syndrome. Up to this moment no false negative was detected.
Limitations, reasons for caution: The main limitation for this test is the cost. The Italian national health system pay only for combined test or, after 35 years old, for invasive tests. The NIPT does not show all the possible chromosomal abnormalities compatible with life. The NIPT+ evidenced a high rate of false positive results.
Wider implications of the findings: Pregnancies achieved through IVF treatments are often describes as "precious". Our results show that in the population (IVF and spontaneous pregnancies) the majority of foetuses are healthy. In fact, only 0.6% (17/2968) tests were positive for aneuploidies and just one for a microdeletion syndrome (0.03%).
Trial registration number: N/A.

P-642 Combined time-lapse imaging and preimplantation genetic screening: a valuable strategy for embryo selection

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Study question: Is the time-lapse tool Eeva useful in a Preimplantational Genetic Screening (PGS) embryo-banking program to select among euploid embryos those with the highest implantation potential?
Summary answer: Eeva Test provides valuable information for embryo selection increasing the chances of pregnancy by accurately predicting the implantation potential of euploid embryos.
What is known already: Previous studies confirmed that genetic screening of human blastocysts significantly improves pregnancy rates per cycle diminishing the likelihood of miscarriage. Unfortunately, there is still a considerable number of euploid embryos that fail to implant. New embryo selection markers to improve ART-outcomes have been developed (mtDNA content, metabolomics or morphokinetic assessment). Time-lapse imaging (TLI) has emerged as an interesting non-invasive tool. No conclusive data about the utility of this technology for chromosome abnormalities determination is available; however, we wonder if combined TLI and PGS embryo selection may be a valuable strategy to identify those embryos with the best chances of success.
Study design, size, duration: This unicentric and retrospective study included 159 embryo-banking PGS-cycles from IVF and egg-donation patients seeking ART treatment at our centre between September 2013 and December 2015. The control group (*PGS-only)* comprised 70 cycles in which embryos had been selected for transfer following euploidy criteria only. The study group (*PGS + Eeva*) comprised 89 cycles in which embryo selection for transfer was based on combined PGS and Eeva predictions.
Participants/materials, setting, methods: All embryos were cultured, biopsied, and vitrified at the blastocyst stage. Genetic analyses of trophectoderm biopsies were performed by NGS. Single euploid blastocyst transfers were performed in all cases. Transfers were differed and under HRT. Within the control *PGS-only* group, the best morphological euploid embryo available was transferred. Within the *PGS + Eeva* group, the euploid blastocyst with the highest Eeva-prediction available was prioritized for transfer. Biochemical pregnancy rates of study groups were statistically compared.
Main results and the role of chance: Clinical characteristics were comparable between groups. There were no significant differences in terms of MII, fertilized eggs, blastocyst rate, high quality blastocyst rate and euploidy rate between the study groups. However, significant differences ($p < 0.05$) were found when comparing pregnancy rates of the control *PGS-only* group [52.9% (37/70)] and the *PGS + Eeva* group cases where euploid embryos with *high* implantation potential as predicted by Eeva were transferred [73.4% (47/64)]. No such differences were found when pregnancy rates from transfers of euploid embryos classified as *medium* or *low* by Eeva were compared to controls. Similar results were obtained when IVF and egg donation cycles were independently analysed. A significantly higher pregnancy rate was achieved in transfers were *high* Eeva prediction in addition to PGS were used for embryo selection [(48% vs 69.2% in IVF cycles ($p < 0.05$) and 54.1% vs 71.9% in egg-donation cycles ($p < 0.05$)]. These results show that Eeva Test provides valuable information for euploid embryo selection significantly increasing the chances of pregnancy in cycles where chromosomally normal embryos predicted by Eeva as *high* are prioritized for transfer.
Limitations, reasons for caution: The retrospective nature of this study may be a reason for caution. Data were collected from one laboratory using a specific culture system and protocols. Moreover, further data regarding clinical outcomes must be included to confirm the predictive value of the analyzed parameters.
Wider implications of the findings: To our knowledge, this is the largest dataset of single euploid embryo transfers selected by an automated time-lapse system. This analysis reveals the ability of early cleavage time-lapse parameters to predict embryo implantation potential and suggest that its combined use with genetic screening of embryos would significantly improve ART outcomes.
Trial registration number: A trial registration number was not required due to the retrospective study design.

P-643 A small trophectoderm biopsy sample is sufficient to detect most mosaicisms after analysis with high resolution next generation sequencing (NGS)

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Study question: Does a biopsy specimen of 4–6 contiguous cells taken from the trophectoderm provide a reliable representation of the remainder of a mosaic human embryo?
Summary answer: When mosaicism was identified in a small trophectoderm biopsy, mosaicism was determined to be present in other embryonic locations in 25/28 (89%) of the embryos.
What is known already: A high percentage of human blastocysts are mosaic. Unlike other methods, it is possible to identify mosaicism in trophectoderm biopsy specimens with high resolution NGS testing. Recent preliminary studies indicate that some mosaic embryos are capable of implantation and normal term development, albeit with a lower implantation rate and higher miscarriage rate than euploid embryos. Little is known of the fate of cell lineages in the human preimplantation embryo after post-meiotic chromosomal malsegregation.
Study design, size, duration: From October to December of 2015, 39 patients underwent aneuploidy screening with NGS, with vitrification of all tested blastocysts. Mosaicism was detected in 48/157 (31%) embryos, alone or in

combination with full aneuploidy. Of these, 28 mosaic blastocysts donated for research were segmented into three sections and fully analyzed by NGS.

Participants/materials, setting, methods: Blastocysts were warmed and those with a well-defined inner cell mass (ICM) were divided into 3 sections with a modified biopsy procedure. First, the ICM was removed without allowing contamination with trophectodermal cells. Then, the trophectoderm was divided into two segments – the embryonic hemisphere, potentially with residual ICM cells, and the abembryonic hemisphere. The specimens were analyzed individually with NGS, and the results were compared to the original biopsy.

Main results and the role of chance: The initial diagnosis of mosaicism was confirmed in all but 3/28 (11%) of the embryos. The overall impact of mosaicism on the rate of false positive misdiagnosis in this group of embryos was low (3/157; 1.9%). While the original diagnosis of mosaic was confirmed in most cases, mosaic cell lines that differed from the original biopsy were frequently detected in one or more of the subsequent biopsies from the same embryo. Only 6/28 (21%) showed identical or substantially similar diagnoses in all 4 blastocyst segments. Mosaicism was confined to either the ICM or the trophectoderm in 11/28 (39%) of blastocysts. In 4/28 (14%) embryos, the ICM biopsy specimen was comprised of only euploid cells, with mosaicism limited to the trophectoderm. There were also 7 embryos (25%) with mosaic ICMs in which only euploid cells were detected in one or both of the trophectoderm hemispheres. None of the 3 types or locations of trophectoderm biopsy was more predictive of ICM mosaicism than the others.

Limitations, reasons for caution: The sample size is small, and should be expanded in order to refine the expectation of occurrence of misdiagnosis. In order to eliminate the possibility of contamination of the ICM during biopsy, the specimen size was sometimes small; a larger specimen may have detected additional mosaicisms.

Wider implications of the findings: The developmental impact of mosaicism limited to either ICM or trophectoderm is unknown, but may be related to decreased implantation rates and increased miscarriage rates observed after transfer of mosaic embryos. The low rate of false positive diagnoses indicates that transfer of mosaic embryos should be undertaken with caution.

Trial registration number: -.

P-644 Association of methylenetetrahydrofolate reductase c677t polymorphism with intervillous and decidual pathology in human cases with pregnancy loss

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Study question: Is there association between methylenetetrahydrofolate reductase (*MTHFR*) C677T polymorphism and intervillous and decidual pathology in patients with pregnancy loss (PL)?

Summary answer: The *MTHFR* C677T TT genotype and T allele are associated with severe intervillous and decidual pathologies in cases with PL.

What is known already: Although an association between PL and TT genotype of the *MTHFR* C677T polymorphism have been well known, the mechanism underlying this association remains obscure. It has been reported increase of placental pathological findings in late PL patients with *MTHFR* C677T TT genotype and another coexisting thrombophilia, but no studies have yet shown that the TT genotype alone could cause this effect. As *MTHFR* C677T polymorphism is common worldwide with at least 13% of the population carrying the TT genotype, the problem is of high importance.

Study design, size, duration: We performed a cross sectional observational study on patients experiencing PL between May 2010 and December 2013. All patients were Japanese ethnicity and had been treated by surgical evacuation. The study was approved by Osaka Medical Center for Maternal and Child Health (MCH) Ethics Committee. The minimum sample size for the study was calculated to be 199 patients and 243 patients were successfully recruited for the study.

Participants/materials, setting, methods: Hematoxylin–eosin stained samples from surgical evacuation were investigated for four pathologies: decidual fibrin (DF), intervillous fibrin (IF), decidual thrombosis (DT), and intervillous thrombosis (IT) and were classified into four degrees – 0, 1, 2, or 3 according to the extent and severity of the pathology. Patients' genotype for *MTHFR* C677T polymorphism was determined by PCR followed by *Hinf*I digestion and gel electrophoresis.

Main results and the role of chance: Out of 300 patients eligible and contacted for the study, 243 patients completed the informed consent and were enrolled into the study, which made a response rate of 81%. From them 83 (34.16%) patients were *MTHFR* C677T CC genotype, 116 patients (47.74%) were CT genotype and 44 patients (18.11%) were TT genotype. Kolmogorov–Smirnov analysis of patients' demographic characteristics showed no significant genotype difference in age, gravity, parity, or gestational age at presentation. There were significantly more T allele carriers [odds ratio (OR) 1.65; $p = 0.032$; 95% CI = 1.041 – 2.614] and TT genotype patients (OR = 2.175; $p = 0.039$; 95% CI = 1.028 – 4.606) among patients with severe (grade 3) IT. Among patients with both grade 3 IT and grade 3 DT, there were also significantly more T allele carriers (OR = 2.412; $p = 0.023$; 95% CI = 1.108 – 5.254) and TT genotype patients (OR = 3.536; $p = 0.021$; 95% CI = 1.148 – 10.892). The CC genotype was protective against the four studied pathologies (OR = 0.533; $p = 0.029$; 95% CI = 0.302 – 0.942). There were no cases without either IF or DF among *MTHFR* C677T TT genotype patients.

Limitations, reasons for caution: As we did not measure serum and red blood cells folate levels, as well as homocysteine levels, it is impossible to establish consecutive chain of events leading to intervillous and decidual pathology in MTHFR C677T T allele and TT genotype carriers. The power of the study was 1.0.

Wider implications of the findings: As *MTHFR* C677T polymorphism is widespread worldwide, our results could suggest a similar study in a country with folate enrichment of cereals, to check the preventive effect of folate on the observed pathologies, as well as a study searching for ultrasound markers based on the observed pathology.

Trial registration number: As the study was not RCT it had not been registered in an ICMJE-recognised trial registry.

P-645 Analysis of DNA from blastocoelic fluid (BF) and corresponding trophectoderm (TE) cells by array-CGH and NGS

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Study question: Can NGS determine the blastocyst chromosome condition through the analysis of DNA from BF and corresponding TE cells in a manner comparable to array-CGH?

Summary answer: NGS provided results on the DNA from BF and corresponding trophectoderm (TE) cells that were quite concordant with those derived by array-CGH.

What is known already: BF contains DNA that can be analyzed by array-CGH, potentially revealing the chromosome status of the corresponding embryo. Previous studies have demonstrated a high level of concordance (97%) between results obtained from TE cells (considered the optimal material for PGS) and those obtained from cytogenetic analysis of BF DNA from the same embryo, suggesting that BF could be used to predict the blastocyst chromosome condition. NGS was recently reported to be more efficient, sensitive and cost-effective than array-CGH. Its use for the study of BF DNA could add valuable information on the predictive capacity of this type of sample.

Study design, size, duration: Cohort study including 49 supernumerary blastocysts, which had been diagnosed by array-CGH on blastomeres from day 3 embryos. BF and corresponding TE cells were then tested to verify whether

the results predicted at the cleavage stage corresponded to the ploidy condition in BFs and TE cells. To confirm the results obtained from the blastocysts, the amplified DNA from 9 BFs and corresponding TE cells was analyzed by NGS by operators blinded to the previous results.

Participants/materials, setting, methods: Nine sets of BFs and corresponding TE cells were selected to undergo NGS. The choice of which samples to evaluate was guided by the following criteria based on the previous array-CGH results: 2 cases of full concordance between blastomere, BF and TE, one of which showed some evidence of mosaicism in BF; 5 cases with segmental abnormalities, including 2 in which mosaicism was suspected; and 2 cases of ploidy discordance between BF and corresponding TE.

Main results and the role of chance: Following reanalysis by NGS, of the two cases with full concordance after array-CGH, 1 was confirmed. The other case (BF and TE were called euploid by array-CGH, although there was some evidence of mosaicism involving 5 chromosomes in the BF) became discordant since the mosaicism suspected in the BF was clearly apparent after NGS; conversely, TE cells were euploid for both diagnostic methods. The 5 cases with segmental abnormalities were all confirmed by NGS, indicating that both techniques are able to identify this type of chromosomal anomalies. Finally, for the cases showing ploidy discordance between BF and TE cells by array-CGH, one remained as such after NGS (BF aneuploid and TE euploid), while the other became ploidy concordant (both BF and TE aneuploid for a single abnormality, although for a different chromosome). In all, NGS confirmed the results provided by array-CGH in 14 of the 16 samples analyzed. The two discrepancies were false-negatives by array-CGH and aneuploid by NGS. Interestingly, in one of these cases, the corresponding TE cells were called euploid by both methods.

Limitations, reasons for caution: BF DNA could derive from any blastocyst compartment, including apoptotic cells. Mosaicism could be present and consequently close attention should be payed to chromosome profiles, especially if using less sensitive methods (e.g., array-CGH). Extending the comparative analysis to more cases could provide additional information on the condition of BF DNA.

Wider implications of the findings: BF samples could be examined using both array-CGH and NGS. Results were concordant in most cases. Two discrepancies were probably due to mosaicism, which is detected more efficiently by NGS. The existence of mosaicism in the BF indicates that the DNA present is sometimes derived from more than one cell.

Trial registration number: Not applicable.

P-646 Fastest benchtop next-generation sequencing workflow for preimplantation genetic screening with Ion ReproSeq™ technology

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Study question: Is the NGS Ion PGM™ System (Thermo-Fisher Scientific) along with Ion ReproSeq™ workflow reliable enough in terms of PGS for aneuploidy and unbalanced translocations detection?

Summary answer: This two-phase validation study demonstrates that Ion ReproSeq™ (Thermo-Fisher Scientific) is more accurate for aneuploidy and unbalanced translocation identification than others technologies and platforms.

What is known already: In PGS, one or more cells from embryos generated through in vitro fertilization (IVF) are removed and tested for overall chromosomal normalcy prior to embryo transfer. PGS was originally performed using fluorescent in-situ hybridization (FISH). This technique showed limitations in genome coverage that were solved by array-based 24sure™ technology with demonstrated accuracy and improved implantation rates. In this way, NGS is the latest breakthrough encouraging method for PGS, offering reliability, higher throughput and personalized assays. *T*he Ion ReproSeq™ PGS Kit with the Ion PGM™ System allows for rapid and affordable screening of aneuploidy and unbalanced translocations in all 24 chromosomes.

Study design, size, duration: This study comprised two phases. Firstly, a double-blinded retrospective validation tested 20 day-5 embryo biopsies in triplicate from 20 IVF cycles by Ion PGM™ platform along with Ion ReproSeq™ PGS as alternative NGS approach, Ion Aneuploidy workflow (Thermo-Fisher Scientific) as traditional NGS method, and array-based 24sure™ Microarrays (Illumina). Secondly, a prospective trial with Ion ReproSeq™ tested 2359 embryo biopsies (92 day-3, 2267 day-5) recruited in 428 clinical IVF cycles from June 2015 to January 2016.

Participants/materials, setting, methods: For retrospective validation, 60 blastocyst biopsies were performed after human oocyte insemination. Prospective study was constituted by 92 blastomeres and 2267 blastocysts biopsies. Whole genome amplification (PicoPlex®, Rubicon-Genomics) preceded traditional NGS and array-based method. CNV analysis for 24sure™ was performed with BlueFuse Multi software (Illumina). The Ion ReproSeq™ consists of whole genome amplification, library construction, isothermal amplification and semiconductor sequencing. Ion Reporter™ Software determined the ploidy status with 0.01X read coverage for both NGS assessments.

Main results and the role of chance: Along retrospective validation, 27 of 60 day-5 embryos resulted euploid, corresponding to 9 different embryos biopsied in triplicate. In respect to aneuploid embryos, 12 of 60 presented monosomies (20%), 9 trisomies (15%), 6 full gain and losses (10%) and 6 had partial imbalances (10%). Along the whole process, no technical failures, false positives and negatives were detected. Almost all triplicates showed the same ploidy status regardless of the PGS technique employed. This first phase provided a 98.3% concordance (99/100; 95% Confidence Interval [CI]: 91.1%-99.9%) for aneuploidy screening by alternative NGS Ion ReproSeq™ in contrast to well-established microarray-based 24Sure™ and traditional NGS Ion Aneuploidy workflow. The discordant result showed a monosomy on chromosome 7 by array, while both NGS approaches detected a partial gain on chromosome 7q. In the prospective study, 2040 of 4718 embryos were euploid; 62 euploid of 184 day-3 and 1978 euploid of 4534 day-5 embryos. A total of 651 aneuploid embryos presented monosomies (24.31%), 630 trisomies (23.52%), 654 full gain and losses (24.42%) and 743 had partial imbalances (27.75%). Moreover, 16 unbalanced translocations were accurately identified from 6 balanced translocation carriers. To date, estimated implantation rate is 60.69% related to 1238 embryos transferred.

Limitations, reasons for caution: Although reliability of NGS Ion PGM™ System along with Ion ReproSeq™ workflow for both aneuploidy and unbalanced translocations detection, further data in a randomized controlled trial are required in order to achieve a broad-based clinical application. Higher throughput would be accomplished by multiplexing more than 24 samples.

Wider implications of the findings: Ion ReproSeq™ is a rapid and cost-effective PGS workflow that provides throughput scalability. Turnaround time is substantially reduced in contrast to around 24 h-protocols (array and previous NGS). In less than 10 h, it's possible to go from 24 embryo biopsies to euploid selection for same day or frozen embryo transfer.

Trial registration number: This is not a clinical trial so it not linked to a trial registration number.

P-647 Chromosomal analysis from multiple biopsies on blastocysts

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Study question: Is there any evidence of mosaicism when testing different biopsies of the same blastocyst?

Summary answer: Mosaicism is present in blastocysts as demonstrated by different results when testing different parts of the same blastocyst.

What is known already: Although at a lower rate compared with cleavage stage embryos, chromosomal mosaicism is a common finding in blastocysts. These anomalies can undergo aneuploidy rescue during following development as demonstrated by the birth of healthy babies from embryos with a low level of mosaicism. As the most currently used method in PGT is trophectoderm (TE) biopsy, it is important to verify to which extent the biopsied TE cells are representative of the blastocyst chromosome status. Recent data have reported the presence of DNA in the blastocoelic fluid (BF) that could be analyzed by array-CGH do determine its chromosome condition.

Study design, size, duration: Cohort study including 9 supernumerary blastocysts, which had been diagnosed as aneuploid by array-CGH on blastomeres from day 3 embryos. BF and corresponding TE cells from two sequential biopsies were then tested to verify the correspondence of the ploidy condition in

BFs and the two TE cell biopsies from each blastocyst. To complete the information, the whole embryo was also tested accounting for a total of 4 analyses for each blastocyst.
Participants/materials, setting, methods: 9 blastocysts from 4 couples in our PGT program were selected for the study. After the aspiration of the BF, blastocysts were left to re-expand. Afterwards, TE cells were sequentially retrieved from two different points of each blastocyst (T1 and T2) following re-expansion of the blastocoelic cavity. BF, T1, T2, and the whole embryo were transferred into separate PCR tubes, submitted to amplification and array-CGH by independent operators. Results were then disclosed and compared.
Main results and the role of chance: Amplified DNA was obtained from 36/36 (100%) samples (9 BFs, 18 trophectoderm biopsies, 9 embryos). Following array-CGH gave only one TE biopsy did not provide an informative result accounting for an efficiency of 97.2% (35/36). In all 9 sets, the analysis of BF correctly predicted the ploidy status of the whole embryo. In 6/8 cases the 2 different TE biopsies gave exactly the same result. In one of the two remaining cases, the ploidy status of the blastocyst was correctly predicted, but the two TE biopsies showed aneuploidy for different chromosomes. In the other case, the results from T1 and T2 were discordant: T2 resulted euploid like the corresponding BF and the whole embryo, while T1 was aneuploid for 3 chromosomes. In both these 2 discordant cases, the profile of whole embryo scan images was not flat suggesting the possibility of mosaicism. In 4/9 cases TE biopsies showed a higher level of mosaicism than BF and the whole embryo. In 2 cases the embryo showed a low level of mosaicism for a segmental aneuploidy, that was detectable also in BF, T1 and T2.
Limitations, reasons for caution: Small size of samples analyzed. Need to validate the results with another analytic method.
Wider implications of the findings: These preliminary data confirm that mosaicism occurs in the blastocyst and this could lead to discordant results and consequently to misdiagnosis. BF, collecting DNA from all blastocyst compartments, could be more representative of the blastocyst ploidy condition.
Trial registration number: Not applicable.

P-648 Genome-wide haplotyping of preimplantation embryos in the clinic: principles guiding embryo selection in Leuven

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Study question: Which are the biological and ethical criteria guiding embryo selection and prioritization following novel genome-wide methods for PGD and which is the clinical outcome?
Summary answer: As guided by the uprise of genome-wide approaches for PGD, a series of selection principles has been has been set and applied clinically.
What is known already: Preimplantation genetic diagnosis by genome-wide haplotyping provides a general overview of the embryonal genome and enable the simultaneous detection of multiple variants, mutations and imbalances genome-wide. The introduction in a diagnostic settings raises novel ethical questions.
Study design, size, duration: From June 2014 until November 2015, 314 embryos from 50 couples seeking PGD for 32 different indications were analyzed in 82 cycles using a novel genome-wide haplotyping-based embryo selection pipeline called siCHILD.
Participants/materials, setting, methods: Patients carrying familial inherited genetic variants and/or chromosomal rearrangements, in risk of obtaining progeny with serious developmental disorders, sought PGD in the University Hospital of Leuven, Belgium. Following consultation with clinical geneticists, fertility specialist and/or counselors, PGD using siCHILD was offered to them. Day 3 embryo biopsy and genetic testing, was followed by embryo selection according to guidelines approved by the local ethical committee. Unaffected embryo were transferred during a fresh or frozen cycle.
Main results and the role of chance: Thirty-two different indications, 28 for monogenic disorders, 3 chromosomal aberrations and 1 case of combined monogenic disorder with chromosomal aberration have been included. Following the analysis of 314 embryos in 82 cycles, at least one embryo was available for transfer in 55 cases. Following embryo transfer, 16 clinical pregnancies (36% clinical pregnancy rate per cycle) and the birth of 5 healthy babies have been achieved so far. No pregnancy was possible in 21 cycles. In 7 cycles, no ongoing pregnancy could be established, due to a biochemical or ectopic pregnancy or an early miscarriage. For the remaining 11 cycles, embryo transfer still needs to be planned. Ranking of embryos based on their carriership status and/or their genome-wide genetic background, has been used in 13 cycles. None of the 5 embryos of low ranking that have been used for transfer resulted in a pregnancy.
Limitations, reasons for caution: siCHILD is applicable whenever haplotype reconstruction is possible, that is the risk allele of interest had been inherited and DNA from family members is available. As novel genome-wide approaches for embryo selection are revolutionizing the field of reproductive genetics, broader discussions to set general guidelines are of outmost importance.
Wider implications of the findings: Our embryo selection principles are based on technical, biological and ethical criteria, have a profound impact on the organization of PGD and the information transferred amongst geneticists, clinicians and patients, are important for the organization of pre- and post-counselling and influence the way of interpreting and reporting preimplantation genotyping results.
Trial registration number: n/a.

P-649 First validation of a next-generation sequencing platform for preimplantation genetic diagnosis of reciprocal translocations using polar bodies

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Study question: Can next-generation sequencing (NGS) accurately detect imbalances associated with reciprocal translocations in first and second polar bodies (PBs), biopsied from human oocytes?
Summary answer: NGS is capable of detecting chromosome abnormalities resulting from the unbalanced segregation of reciprocal translocations in PBs and offers significant advantages over established PGD methods.
What is known already: Recently there has been much interest in using NGS for the cytogenetic assessment of embryos. Several studies have described the validation and clinical application of different NGS approaches for the analysis of cells biopsied from cleavage and blastocyst stage embryos in the context of preimplantation genetic screening (PGS). NGS protocols are reported to have cost and accuracy advantages over other methods. It is unclear, however, whether NGS can be employed for the preimplantation genetic diagnosis (PGD) of reciprocal translocations. It is also unknown if NGS can reliably assess the chromosome complement of first and second PBs biopsied from oocytes.
Study design, size, duration: First and second PBs were biopsied from 54 oocytes. The oocytes were derived from a total of 24 women undergoing IVF who were carriers of different reciprocal translocations. All PBs were classified as abnormal after microarray comparative genomic hybridization (aCGH) analysis. These PBs were subsequently re-analysed via an NGS platform. This analysis took place in a blinded manner.
Participants/materials, setting, methods: All biopsied PBs initially underwent a whole genome amplification approach, producing microgram quantities of DNA. The DNA generated was initially employed for comprehensive chromosome assessment using a well-established aCGH approach. Subsequently, a second aliquot of the amplified DNA was utilized for the purpose of NGS. Sequencing was focused on enumeration of chromosome copy number only, specific gene sequences were not investigated.
Main results and the role of chance: NGS succeeded in yielding results for all 54 first and second PBs, and reliably identified all unbalanced reciprocal translocation forms. The smallest unbalanced fragment assessed was ~2–5 Mb, indicating that the NGS resolution is at least as good as aCGH. The data obtained revealed that abnormal segregation of reciprocal translocations occurred most frequently during meiosis I (MI) (80%), although there were cases where translocation chromosomes segregated abnormally during meiosis II (MII) (9%), or during both meiotic divisions (11%). NGS was seen to have a greater dynamic range than aCGH, allowing single chromatid losses/gains to be readily distinguished from errors involving entire chromosomes in first PBs. We

were therefore able to determine that 59 of the 103 (57%) translocation-related structural abnormalities were due to single chromatid malsegregation, with the remaining 44/103 (43%) associated with entire chromosome malsegregation. Correction during the second meiotic division of translocation-related errors that had occurred in MI was observed in 21/54 (39%) second PBs, leading to a total of 6 (11%) chromosomally normal oocytes. NGS detected an additional 48 MI abnormalities that were unrelated to the translocation (29 involving chromatids and 19 affecting entire chromosomes) and 22 MII abnormalities. Comparison of NGS and aCGH showed a 96% concordance.

Limitations, reasons for caution: Assessment of a larger number of first and second PBs from translocation carriers will provide additional assurance that NGS can be reliably employed for the cytogenetic analysis of this type of cell. Assessment of an even greater variety of translocations will help to further define the resolution of the method.

Wider implications of the findings: Our findings indicate that NGS can accurately detect unbalanced products of reciprocal translocations in first and second PBs. The method was highly sensitive enabling accurate distinction between single chromatid and entire chromosome errors, and determined that translocation malsegregation affects chromatid sites more frequently than chromosome ones.

Trial registration number: N/A.

P-650 Validation of next generation sequence (NGS) for PGD of structural chromosome abnormalities

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Study question: Can the VeriSeq PGS NGS assay detect unbalanced structural chromosome abnormalities and provide higher analytical depth in comparison to array comparative genomic hybridization (aCGH)?

Summary answer: NGS is capable of detecting unbalanced structural chromosome abnormalities in embryos and proves to have higher resolution than aCGH.

What is known already: Structural chromosome abnormalities in embryos were first detected via fluorescence *in situ* hybridization (FISH). In addition to being unable to test for the full complement of chromosomes, FISH required probe preparation following exhaustive analysis for each particular case. Array-CGH was validated for structural chromosome abnormalities and provided the advantage of being able to screen for aneuploidy of all the chromosomes while simultaneously detecting the translocation, typically without prior test preparation. However, aCGH cannot be used if 2 out of the 4 chromosome fragments involved in the translocation are below 6 Mb; therefore, FISH is still necessitated for some cryptic translocations.

Study design, size, duration: 75 embryos were reanalysed via Veriseq PGS Solution comprising of 300 regions of the genome involved in translocations. Since the amplification method for both array-CGH and NGS is identical, there was no need to rebiopsy any embryos. An aliquot consisting of 5 ul was used from the previously analysed array-CGH samples consisting of known structural chromosome abnormalities. A blinded analysis was then performed to evaluate the results.

Participants/materials, setting, methods: SurePlex whole genome amplification products from trophectoderm biopsies that were previously performed to evaluate for a known structural chromosome rearrangement by aCGH were processed using the Illumina VeriSeq PGS assay with the MiSeq sequencing platform. Results were interpreted through BlueFuse Multi analysis software.

Main results and the role of chance: NGS analysis provides accurate information regarding regions involved in the translocation because it can detect copy numbers of those regions ranging from 0 to N copies. NGS analysis revealed a higher resolution compared to aCGH by accurately detecting unbalanced embryos when the regions involved in the translocations were 3 Mb or above in 100% of segments 3–6 Mb (n = 39). The size of the breakpoints of the 75 embryos ranged from 1.6 Mb to 164.0 Mb. These embryos had been diagnosed as either "Unbalanced" with any gain or loss associated with chromosomes involved in the translocation or "Normal or Balanced". 74/75 samples were concordant with the analysis (98.67%). The discordant sample was a triploid embryo, which is undetectable by aCGH. Of the chromosomal regions involved in the translocations, 271/300 were concordant, and 29 were discordant. These 29 discordances were due to the inability of the aCGH technique to detect gains or losses in of less than 6 Mb, inaccuracy in giving the correct copy number, or mosaicism.

Limitations, reasons for caution: NGS does not allow the distinction between normal or balanced embryos; this is also a limitation of aCGH and FISH testing. Additionally, if two or more fragments involved in the translocation have a size less than 3 Mb, NGS would not be recommended.

Wider implications of the findings: This advancement can provide subtle structural chromosome rearrangement carriers the opportunity to use NGS over FISH so they can screen for all chromosomes and the detection of polyploidy. FISH is limited because it requires fixation of blastomeres whereas NGS allows for analysis of trophectoderm samples from a blastocyst biopsy.

Trial registration number: N/A.

P-651 Multinucleated embryos: to transfer or not to transfer?

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Study question: Is it justified to transfer multinucleated embryos although they have a reduced developmental potential and an increased rate of chromosomal abnormalities?

Summary answer: Multinucleated embryos show an altered morphokinetic pattern and a decreased developmental potential, however euploid blastocysts from multinucleated and non multinucleated embryos have similar implantation capacity.

What is known already: Multinucleation is a common phenomenon in *in vitro* human embryos and is associated to an impaired developmental capacity and increased chromosomal abnormalities. Time-lapse monitoring of embryo development allows a continous and detailed observation of fertilization and cleavage events. Multinucleated embryos are not usually transferred unless non multinucleated embryos are available.

Study design, size, duration: Retrospective analysis of 4192 embryos cultured in a time-lapse system from 534 ICSI cycles and 1908 embryos cultured in a time-lapse system from 199 PGS cycles performed between 2012 and 2014.

Participants/materials, setting, methods: Embryos were cultured in an Embryoscope incubator after ICSI. Presence of multinucleation in all developmental stages was retrospectively assessed. Embryo replacement was performed on D + 3. Multinucleated embryos were cultured to the blastocyst stage and cryopreserved for subsequent transfer.

In PGS cicles, embryos were biopsied on D + 3. One cell was analysed by a-CGH. Euploid embryos were transferred and/or cryopreserved at the blastocyst stage. Correlations between multinucleation and morphokinetics, developmental ability, chromosomal constitution and implantation capacity were established.

Main results and the role of chance: Twenty three point six percent of the studied embryos showed multinucleation. Multinucleated embryos presented delayed morphokinetic behaviour and reduced developmental capacity. Eighteen per cent of multinucleated embryos reached the blastocyst stage and were cryopreserved. The survival rate after thawing was 84.6%. From multinucleated KID replacements, the implantation rate was 39.1%.

The percentatge of embryos biopsied did not differ between multinucleated and non-multinucleated embryos (93.1% vs. 95.0%). Multinucleated embryos showed a lower euploidy rate (14.0% vs. 21.3%), and a higher percentatge of complex aneuploidy (58.2% vs. 45.4%) when compared to non multinucleated ones. No differences were observed in the percentage of aneuploidy with respect to the multinucleation type (81.1% binucleated vs. 85.5% multi/micronucleated) or percentatge of multinucleated cells (82.7% for ≤50% vs. 86.9 for >50%). Similar implantation rates were observed after transfer of euploid blastocists derived from multinucleated or non multinucleated embryos in 75 KID-PGS transfers.

Limitations, reasons for caution: In ICSI cycles, non multinucleated embryos were only cultured to D + 3 and thus do not represent a control group for the multinucleated embryos with respect to the blastocyst rate.

In PGS cycles, the chromosomal constitution has been assessed through one cell biopsy and thus embryo mosaicism could not be evaluated.
Wider implications of the findings: Multinucleated embryos that reach the blastocyst stage can be replaced and present a high implantation potential.
Trial registration number: –.

P-652 Preimplantation genetic diagnosis (PGD) for inherited disorders using Karyomapping with optional preimplantation genetic screening (PGS): diagnostic and clinical outcomes from 576 cycles

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Study question: Is PGD using Karyomapping, a universal single nucleotide polymorphism (SNP)-array linkage analysis technique, a successful treatment strategy for patients requesting embryo testing for inherited disorders?
Summary answer: High accuracy diagnostic and implantation rates were observed with faster time to diagnosis/pregnancy. The majority of cases involved combined PGD + PGS and single embryo transfer.
What is known already: Karyomapping utilizes a universal protocol for linkage-based PGD applicable to virtually all patients. It eliminates the need to develop and optimize patient-specific protocols, drastically reducing the waiting time for initiation of IVF treatment. This technology was previously validated using the gold-standard polymerase chain reaction methodology (with 100% concordance) and has proven to be highly efficient for even the most complex of cases, including those not feasible using conventional PGD methods, such as deletions and duplications. Additionally, it is possible to combine Karyomapping with PGS using microarray comparative genome hybridization (aCGH) or next generation sequencing (NGS).
Study design, size, duration: Among the 576 Karyomapping cases performed between January 2014 and January 2016, clinical outcomes and follow-up data was collected for 447 PGD cycles with embryos suitable for transfer. Two hundred and seven cycles had undergone embryo transfer at the time of data collection, while the rest remain frozen or had no euploid and disease free embryos for transfer.
Participants/materials, setting, methods: A total of 3584 embryos were biopsied at the blastocyst stage and frozen for transfer in subsequent cycle(s). Samples were whole genome amplified and analyzed using Karyomapping. Additionally, most patients (87%) requested PGS, which was performed independently using aCGH (462 cases) or NGS (40 cases).
Main results and the role of chance: PGD was offered for over 90 different single gene disorders, deletions/duplications or translocations (10 different cases) and/or HLA-matching (14 cases). The Karyomapping diagnostic rate was 96.7%. The average numbers of embryos analyzed and eligible for transfer per cycle were 4.75 and 2.12 for cases undergoing PGD alone and 6.3 and 1.8 for PGD + PGS.

The implantation rate was 74.3% (135/183) for PGD + PGS (average maternal age: 34.1 years) and 66.7% (16/24) for PGD alone (average maternal age: 32.5 years). The proportions of cycles with transferable embryos and average number of transfers per cycle started were similar for PGD + PGS (75% and 0.98) and PGD alone (76% and 1.12). Most transfers involved a single thawed blastocyst, except for seventeen patients (9.4%) who had two embryos replaced together.

There were 23 healthy live births and 82 ongoing pregnancies at the time of analysis. Prenatal and perinatal testing when performed confirmed PGD in all instances. No misdiagnoses have been reported to date (0/105 misdiagnosed pregnancies).
Limitations, reasons for caution: The collection of clinical outcome and follow-up data can be challenging, particularly when dealing with large numbers of referring centers and overseas patients. Few patients elect to undergo prenatal testing to confirm the diagnosis. Ongoing follow-up data analyses will conclusively reveal the high accuracy of PGD using Karyomapping.
Wider implications of the findings: Karyomapping allows PGD laboratories to cope with the rapidly growing demand for embryo testing and diagnose a wider range of disorders. The high implantation rates were similar to outcomes achieved by the most successful *in vitro* fertilization programs. Simultaneous PGS speeds up the testing process and further improves clinical outcomes.
Trial registration number: Not applicable.

P-653 Abnormal serum dehydroepiandrosterone sulphate (DHEAS) concentration in *in vitro* fertilization patients increased embryo aneuploidy assessed by next-generation sequencing technology (NGS)

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Study question: To assess correlation between serum hormones concentration, ovarian reserve and ploidy status in *in vitro* fertilization patients.
Summary answer: Increased serum DHEAS concentration in women without DHEA suplementation increases the risk of aneuploid embryos compared to patients with normal serum DHEAS.
What is known already: Dehydroepiandrosterone (DHEA) has been demonstrated to improve embryo quality and pregnancy chances in women with diminished ovarian reserve (DOR). It is still unknown, how these effects are achieved. DHEA supplementation reduces aneuploidy in women with DOR.
Study design, size, duration: Retrospective study including the data analysis of 163 IVF/PGS cycles (median age 38 years, range 34–40) performed between January 2014 and September 2015 at INVICTA Fertility Centre, Poland. A total of 561 blastocysts were evaluated with the NGS protocol. No DHEA supplementation was used before IVF cycles. Only first IVF cycles were analysed.
Participants/materials, setting, methods: Normal serum DHEAS concentrations were found in 144 patients, higher in 19. The embryo ploidy status was assessed by dividing number of aneuploid embryos by the numbers of normal embryos. Two PGS groups were analysed: one with majority of aneuploid embryos (factor >1 of aneuploidy vs. normal embryos) and second with majority of normal blastocysts (factor ≤1 of aneuploidy vs. normal embryos).
Main results and the role of chance: Two PGS groups did not differ in age, serum AMH, InhibinB, TSH, T, SHBG concentration. 93 (64.6%) patients with normal serum DHEAS had advantage aneuploidy, while in the group with a higher serum DHEA rate of aneuploidy was statistically significant higher ($p = 0.05$) and amounted 84.2% (16 patients). The univariate regression analysis of variables studied for the prediction of advantage aneuploidy showed that serum DHEA is a predictor for advantage aneuploidy (OR 3.27; 95% CI, 1.06–10.12). The risk of increased advantage aneuploidy in all analysed embryos from one cycle is more than three times higher in women with increased serum DHEAS concentration ($p = 0.01$).
Limitations, reasons for caution: The study is limited by sample size. A higher sample size or a prospective randomized design could be used in future studies to corroborate the current findings.
Wider implications of the findings: Increased serum DHEAS concentration in *in vitro* fertilization patients may be an indication to perform PGD additionally. The risk of aneuploidy is higher in patient with increased serum DHEAS concentration than in normal.
Trial registration number: Not applicable.

P-654 Why do euploid embryos miscarry? A retrospective study comparing aneuploidy rates within presumed euploid embryos resulting in miscarriage or live birth using next-generation sequencing (NGS)

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Study question: Does undetected aneuploidy or mosaicism contribute to pregnancy loss after transfer of euploid embryos by array comparative genomic hybridization (aCGH)?
Summary answer: NGS detects more cases of mosaicism and triploidy than aCGH, and mosaicism rates are significantly higher among pregnancies resulting in miscarriage than live birth.
What is known already: Array CGH is widely used for pre-implantation genetic screening (PGS). NGS is capable of detecting more cases of mosaicism and triploidy (69XXY), which may assist in reducing the incidence of spontaneous abortion and increase ongoing pregnancy rates.

Study design, size, duration: Retrospective study of 183 patients undergoing PGS by aCGH between 8/2012 and 5/2015 at New York University Fertility Center and Oregon Reproductive Medicine.
Participants/materials, setting, methods: Saved amplified DNA samples from the 183 blastocyst trophectoderm (TE) biopsies previously diagnosed as euploid by aCGH were re-analyzed using the miSeq NGS platform (Illumina, USA) and VeriSeq NGS technology (Illumina, USA). 44 embryos resulting in a biochemical pregnancy, 62 resulting in miscarriage, and 77 resulting in live birth were available for re-analysis.
Main results and the role of chance: 25% (11/44) of embryos resulting in biochemical pregnancies were mosaic, and one embryo was found to be triploid (69, XXY) by NGS. 33.9% (21/62) and 3.2% (2/62) of embryos resulting in miscarriage were mosaic and triploid by NGS, respectively. In contrast, the mosaicism rate among embryos resulting in live birth was only 13% (10/77), which was significantly lower than the rate of mosaicism among miscarriages ($p = 0.0062$, RR 1.78 with 95% CI 1.23–2.5).
Limitations, reasons for caution: This study was limited by its retrospective design. Up to 10% of DNA samples that were undergoing re-analysis were excluded due to degraded DNA, although the frequency was similar in all three groups.
Wider implications of the findings: Undetected mosaicism may increase the risk of first trimester pregnancy loss. NGS is more sensitive at picking up mosaicism and triploidy than aCGH. Mosaic embryos can be considered for transfer after genetic counseling and informed consent, but they have a higher miscarriage rate as well as unknown post-natal genetic effects.
Trial registration number: N/A.

P-655 Utilizing CGG repeat length to determine Anti-Müllerian Hormone (AMH) levels in women without the Fragile X premutation

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Study question: Is there a correlation between the number of CGG repeats on the FMR1 gene and AMH values in women without the Fragile X premutation?
Summary answer: Our data set, the largest to date, shows that repeats deviating from the normal range, 29/30, correlate with decreased AMH levels.
What is known already: The number of CGG repeats on the Fragile X (FMR1) gene has been known to correlate with premature ovarian failure in patients with the premutation (CGG repeats of 55–200). Previous studies have discussed the relationship between CGG repeats less than 45 and diminished ovarian reserve using serum AMH levels, as AMH has been shown to be an accurate biomarker for ovarian reserve. Yet, the relationship remains inconclusive based on conflicting results.
Study design, size, duration: A retrospective cohort study of 1842 infertility patients seen at our University affiliated fertility center between June 2013 and July 2015.
Participants/materials, setting, methods: CGG repeats on allele 1, allele 2 and the average of both alleles were obtained as well as serum AMH values. The cohort was stratified based on age, (≤35 ($n = 936$), >35 ($n = 888$)) and further stratified based on the number of repeats (<29, 29/30, >30). Patients with AMH >10 and CGG repeats of 45–55 (grey zone) were excluded. AMH values of patients with CGG repeats <29 and >30 repeats were compared to the normal range of 29/30.
Main results and the role of chance: Allele 1 repeats <29 yielded lower AMH values than the normal repeat pattern in both the ≤35 and >35 age groups ($p = 0.03$, $p = 0.01$, respectively). Allele 1 repeats >30 in the >35 age group yielded lower AMH values ($p = 0.01$). Allele 2 repeats >30 in the ≤35 aged women yielded lower AMH ($p = 0.04$). When the average of both alleles was <29 in both younger and older age groups, AMH was decreased. ($p = 0.08$, $p = 0.01$, respectively).
Limitations, reasons for caution: All laboratory investigations were performed using commercial assays. (Counsyl, San Francisco, CA.)
Wider implications of the findings: Our findings are consistent with prior studies showing that deviations from the normal 29 and 30 CGG repeats on allele 1 and allele 2 correlated with decreased AMH. Departure from DNA's normal stability may cause fluctuations in patients' fertility profiles. Larger scale studies will be beneficial to confirm these results.
Trial registration number: NA.

P-656 Euploid and aneuploid rates on day 5 vs. day 6 of blastulation

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Study question: Is there a higher rate of aneuploidy in blastocysts biopsied on day 5 compared to day 6?
Summary answer: Increased aneuploidy rates were observed on day 6 biopsied blastocysts compared to day 5 in patients less than 35-year-old.
What is known already: Comprehensive Chromosomal Screening (CCS) is used for aneuploidy analysis at different phases of embryo development. In the past decade, there has been a shift from cleavage stage biopsies to blastocyst biopsies, believed to be more reliable (less chance of mosaiscm) and associated with higher implantation rates. It is believed that embryos that are slower to progress (day 6) have a higher chance of Meiosis I and II non-disjunction, compared with faster developing embryos (day 5). Previous studies have indeed demonstrated an increased rates aneuploidy in day 6 biopsied embryos compared with day 5.
Study design, size, duration: Retrospective cohort study of 58 patients (69 cycles) who underwent IVF between April 2014 and December 2015 with a single physician at an University affiliated academic institution in New York City.
Participants/materials, setting, methods: Results obtained for embryos from patients who underwent CCS were reviewed. 358 embryos met the inclusion criteria, were biopsied as they reached the blastocyst stage and vitrified for future transfer. CCS was completed by Reprogenetics® (New Jersey) by array comparative genomic hybridization (aCGH) to determine euploid or aneuploid status. Euploidy and aneuploidy rates were then compared on day 5 vs. day 6 biopsy. In addition, aneuploidy rates were compared when controlled for age (≤35 and >35).
Main results and the role of chance: 358 embryos were included in the study. 110 were biopsied on day 5 and 248 were biopsied on day 6. Euploid rates was higher for day 5 embryos (61/110, 56%) compared to day 6 (111/248, 45%) ($p = 0.06$). When stratifying by age, euploid rates of day 5 embryos in the ≤35 age group were 75% (41/55), significantly higher than the euploid rate on day 6, 59% (63/107) ($p = 0.04$). In the >35 age group, the aneuploidy rates on day 5 (35/55, 63%) and day 6 (93/141, 66%) were not different statistically ($p = 0.71$).
Limitations, reasons for caution: Not applicable.
Wider implications of the findings: In younger patients undergoing CCS, day 5 embryos have significantly higher euploid rates compared with day 6. Larger studies are needed to support the data.
Trial registration number: NA.

P-657 Skewed X-inactivation and telomere content in premature ovarian failure: a preliminary study

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Study question: X-inactivation pattern and telomere content are related events in premature ovarian failure (POF)?
Summary answer: Telomere content was reduced in POF women that also presented skewed X inactivation profile.
What is known already: Anovulation in POF women can be related to structurally aberrant X-chromosomes or 45, X/46, XX mosaics, in which X chromosome inactivation (XCI) pattern could represent an epigenetic marker in cryptic mosaicism. Deregulation of epigenetic control, such as nonrandom X inactivation in female genome is related to telomere dysfunction and maybe an important feature of POF.
Study design, size, duration: Case–control study, in which 34 non-syndromic POF and 75 controls with regular menstrual cycle were included.
Participants/materials, setting, methods: POF (FSH > 40 IU/l) with 46, XX karyotype and control women with regular menstrual cycle (FSH < 10 IU/l) participated in this study. Height, age, weight, body mass index (BMI), and systemic (SAP) and diastolic arterial pressure (DAP) were measured. Telomere

content was measured using quantitative real time PCR (qPCR). XCI analysis was based on human androgen receptor (HUMARA) and X-linked retinitis pigmentosa 2 (*RP2*) assays. Statistical analyses were determined by Student's *t*-test and chi-square test (χ^2).

Main results and the role of chance: No statistical differences were observed between POF and controls in age, height, BMI, SAP and DAP. The POF group presented less weight than controls (65.03 ± 12.48 and 72.95 ± 18.53; $P = 0.02$). POF group showed reduced telomere content (0.90 ± 0.21; $n = 33$) when compared to control group (1.07 ± 0.29; $n = 75$) ($P = 0.01$). Skewed XCI (≥75%) was more frequent in POF (42.42%, $n = 14$) than control (15.8%, $n = 3$) group ($P = 0.02$). Random XCI was observed in 54.55% ($n = 18$) of POF and 86.49% ($n = 34$) of control group. XCI could not be detected in only one case of POF (3.03%) that was homozygote for both markers. Interestingly, from those POF women with skewed XCI, extreme skewing (≥90%) was observed in 50% ($n = 7$).

Limitations, reasons for caution: Limited number of cases of the study group (POF). An increase of sample size is recommended to make the data more robust and confirm the results.

Wider implications of the findings: Skewed XCI and reduced telomere content was observed in POF women, a condition that is maybe correlated. Also, extreme skewing is a rare phenomenon in the normal female population but is frequently observed in X-linked mutations and telomere dysfunction, suggesting a possible role in POF etiology.

Trial registration number: N/A.

P-658 Whole genome sequencing analysis reveals genes related to lipid metabolism and inflammation are disrupted in women across different infertility diagnoses

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Study question: Reproductive disorders, such as endometriosis and PCOS, share common phenotypes, such as increased miscarriage rates. We aimed to understand whether disruptions in the same molecular pathways underlie these common phenotypes.

Summary answer: Disruptions in genes playing a role in lipid metabolism and inflammation are frequently associated with a variety of reproductive phenotypes in patients undergoing fertility treatment.

What is known already: Research aimed at decoding the genetic basis of infertility phenotypes has typically focused on single genes or regions in the context of single diagnoses. For example, many studies demonstrate an association between inflammation and endometriosis, and studies focusing on PCOS often look at lipid metabolism. However, no studies have embarked on genome wide interrogation of the spectrum of reproductive disorders.

Study design, size, duration: We first built a genomic knowledgebase by performing a systematic literature review of 20,000 publications linking a genotype to a reproductive outcome. Then, we collected blood samples from 253 women with different diagnoses undergoing treatment at 5 fertility centers between November 2012 and March 2015. Women with a natural parity of ≥1, history of gynecological surgery, cancer treatment, genetic syndromes or major illness or trauma that would compromise fertility, were excluded.

Participants/materials, setting, methods: After obtaining IRB consent, blood samples were collected from all patients and whole genome sequencing performed on extracted DNA using Illumina sequencers. Bioinformatics tools were then applied to report variant calls, novel and deleterious variants. Patient variant signatures were then analyzed using phenotypic and genotypic data curated from our knowledge base.

Main results and the role of chance: Our analysis reveals that a relatively small number of genes recur in their association with multiple fertility-related pathologies. For example, deleterious mutations in GSTP1 and MALRD1 occur between 2.6 and 6.6 times more frequent in both endometriosis and PCOS patients than in the general population. These genes are known to play roles in the oxidative stress response and lipid metabolism, both of which contribute to hormonal regulation and inflammatory response mechanisms in the ovary and endometrium. Our results suggest that fertility-related pathologies can be associated with genetic variants among both diagnosis-specific genes and pathways, as well as a core set of variants common to all or many reproductive phenotypes. Such insight could facilitate the identification of patients at risk of infertility through conditions that we understand less, or that require more invasive diagnostic procedures.

Limitations, reasons for caution: While the biological effect of many genetic variants is well-established, epistatic effects of these variants on human fertility is not fully understood.

Wider implications of the findings: A genomic approach to reproductive dysfunction may aid in molecular characterization of disease, and when combined with the classical clinical phenotypic approach, may improve both diagnostic and therapeutic accuracy.

Trial registration number: None.

P-659 Aneuploidy screening on polar bodies: next generation sequencing versus array comparative genomic hybrization

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Study question: Can whole chromosome- and segmental aneuploidies be detected by Next Generation sequencing (NGS) in polar bodies? How is the result concordance between array comparative genomic hybrization (aCGH) and NGS?

Summary answer: NGS is able to detect aneuploidies with high accuracy and concordance compared to aCGH. NGS appears to be a robust methodology for 24-chromosome aneuploidy screening.

What is known already: Preimplantation genetic screening (PGS) improves implantation rate, decreases miscarriage rate and decreases the risk of abnormal offspring. PGS methodologies evolved from fluorescence *in situ* hybridization to aCGH and finally to NGS.

Study design, size, duration: NGS based aneuploidy screening was evaluated by analyzing pretested polar bodies. Therefore, 22 clinical polar bodies, which were screened by aCGH between November and December 2015, were selected. Selection criteria were based on clear or borderline whole chromosome- or segmental aneuploidies.

Participants/materials, setting, methods: 1 ng of whole genome amplification products were used NGS based aneuploidy screening (VeriSeq PGS, Illumina). Library preparation consisted of photometric quantitation, tagmentation and amplification of dual indexed fragments. Each library was normalised before pooling and loaded into a MiSeq instrument (Illumina, USA).

Main results and the role of chance: Overall, great concordance was observed between NGS and aCGH. All single, dual and complex (≥3) aneuploid samples were confirmed by NGS. 2 segmental aneuploidies were confirmed and detected with a much greater resolution compared to aCGH. Importantly, all polar bodies diagnosed as euploid by array-CGH were confirmed as euploid with NGS and *vice versa*.

Limitations, reasons for caution: Although NGS based PGS exhibited high concordance with aCGH for both numerical and structural chromosome abnormalities, further randomized control trials subsequent clinical validation studies are required to better define the sensitivity, specificity and positive and negative predictive values of NGS based polar body aneuploidy screening.

Wider implications of the findings: There were additional advantages with use of NGS, the high resolution NGS based PGS might be especially important for mosaicism detection. Additionally, it offers also the analysis of multiple samples from multiple patients with different indications on the same platform. Despite these advantages one major drawback of NGS

Trial registration number: N/A.

POSTER VIEWING SESSION

REPRODUCTIVE ENDOCRINOLOGY

P-660 Aberrant expression and DNA methylation of lipid metabolism genes in PCOS: a new insight into its pathogenesis

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Study question: The transcriptone profiling of granulosa cells (GCs) and related differential methylation in polycystic ovary syndrome (PCOS) remain to be illustrated.
Summary answer: The hypomethylated genes related to synthesis of lipid and steroid might dysregulate the expression of these genes and promote synthesis of steroid hormones including androgen.
What is known already: PCOS, characterized by chronic anovulation and hyperandrogenism, is one of the most prevalent endocrine disorders in women of reproductive age. The pathogenesis of PCOS is not fully illustrated, and several lines of evidence demonstrate that PCOS is a complex and multifactorial disorder with a high degree of heritability. The high degree of familial aggregation of PCOS suggests that genetic factor plays an important role in its etiology, and recent genetic studies have also been performed with not much added information to the field.
Study design, size, duration: Seventy-one women with PCOS and fifty women with normal ovulatory cycles (control) undergoing *in vitro* fertilization during September 2014–June 2015 participated in this case–control study.
Participants/materials, setting, methods: The subjects enrolled in this study from these two groups have comparable BMI levels and age. RNA-Seq was performed using Illumina Hiseq 2000. The transcriptome results for selected genes were confirmed by real-time quantitative PCR. 5-methycytosine analysis and MassArray EpiTYPER quantitative DNA methylation analysis were employed to determine the methylation level of global DNA and each CpG site or unit in the promoters of related genes, respectively.
Main results and the role of chance: RNA-seq identified 92 DEGs in PCOS GCs in comparison with the corresponding controls. Bioinformatics analysis indicated that synthesis of lipid and steroid was activated in PCOS GCs. 5-Methycytosine analysis showed that there was an approximate 25% reduction in global DNA methylation of GCs from PCOS women (4.44 ± 0.65%) compared with the controls (6.07 ± 0.72%, $P < 0.05$). Using the introduction of MassArray EpiTYPER quantitative DNA methylation analysis, we also found the hypomethylation in the promoters of several genes related to synthesis of lipid and steroid, which might result in the abberant expression of these genes.
Limitations, reasons for caution: A sample size of $n = 3$ for RNA seq study is inadequate. This could inevitably cause some false positive and false negative results. We validated some of the positive results, and got consistent results.
Wider implications of the findings: The hypomethylated alterations in follicular cells may results in permanent modifications of the offspring's epigonome and alterations of their gene-expression patterns, which may increase susceptibility of adult diseases.
Trial registration number: None.

P-661 The "normal" range of FMR1 triple CGG repeats may be associated with primary ovarian insufficiency in China

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Study question: What is the correlation between normal numbers of Fragile X mental retardation gene 1 (*FMR1*) CGG repeats and POI occurrence or subsequent resumption of ovarian function.
Summary answer: Fewer than 26 or more than 28 CGG repeats CGG repeat number in the smaller allele (allele1) of *FMR1* were significantly associated with POI occurrence.
What is known already: *FMR1* premutation was not a common explanation for sporadic POF in Han Chinese. Also, few studies have investigated the correlation between normal range of *FMR1* CGG repeats number and Chinese POI occurrence and no data on subsequent resumption of ovarian function.
Study design, size, duration: A retrospective study was performed at our center for reproductive endocrinology. A total of 122 occult POI cases and 105 controls were followed up and analyzed from 2012 to 2014 in our center.
Participants/materials, setting, methods: The number of *FMR1* CGG repeats were detected by capillary electrophoresis for each patient. The correlation between CGG repeats and the occurrence of POI or the resumption of ovarian function was evaluated by Chi-squared test or logistic regression analysis.
Main results and the role of chance: The prevalence of premutation and intermediate range of *FMR1* in Han Chinese POI patients was only 0.81% (1/122) and 1.64% (2/122), respectively. The risk of POI occurrence for <26 CGG repeats in allele1 was significantly higher than that for 26–28 CGG repeats ($P < 0.001$, odds ratio 13.50, 95% confidence interval: 3.21–56.77). The risk of POI occurrence for ≥29 CGG repeats in allele1 was also higher than that for 26–28 CGG repeats ($P < 0.001$, odds ratio 6.07, 95% confidence interval: 2.18–16.90). Furthermore, patients with a history of pregnancy had a higher ovarian function resumption rate (45.45% vs. 10.19%; Fisher, $P = 0.007$). However, there was no significant difference in the CGG repeat distribution (<26, 26–28, or ≥29) in *FMR1* allele1 between POI cases whose ovarian function resumed and those whose ovarian function did not.
Limitations, reasons for caution: The study focuses on the relationship between number of *FMR1* CGG repeats and POI occurrence or ovarian function resumption, but is absent of the mechanism study on why ovarian disturbance is so sensitive to small changes in CGG repeats.
Wider implications of the findings: The *FMR1* gene plays a more significant role in POI than was previously thought. Allele1, but not allele2, plays a major role in POI risk prediction. Fewer than 26 or more than 28 CGG repeats in *FMR1* allele1 may be used to predict the probability of POI occurrence.
Trial registration number: no.

P-662 Comparison of results treated with double stimulation protocol and short downregulation protocol for ovarian stimulation in poor ovarian responder: a prospective matched cohort study

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Study question: What kind of protocol is better for ovarian stimulation in poor ovarian responder (POR)?
Summary answer: Double stimulation is a safe, patient-friendly, cost effective protocol for POR and relates good quality embryos.
What is known already: A dramatic lack of antral follicles contributes to reduced oocyte/embryo quantity and quality in POR. Double stimulation protocol provides two opportunities for retrieving oocytes in one menstrual cycle.
Study design, size, duration: From July 2014 to December 2015, a prospective matched cohort study recruited 300 patients were performed in an academic center for reproductive medicine.
Participants/materials, setting, methods: In the academic fertility center, POR were recruited in their first *in vitro* fertilization (IVF) cycles. Matched for age and body mass index, these 300 patients were divided randomly into two groups. After ovarian stimulation with different protocol, the stimulation parameters, accumulated clinical pregnancy rate and embryo implantation rate were compared.
Main results and the role of chance: By the end of the study, 266 patients (133 couples) reached to the stage of follicular puncture and accepted follow-up. The risk of failure in oocyte retrieval was higher in double stimulation protocol (6.02% vs. 0.75%, $p = 0.036$). No significant difference was detected between the two protocols regarding the number of retrieved oocytes (3.22 ± 2.60 vs. 3.03 ± 1.71, $p = 0.784$). But lower dose of exogenous gonadotrophin was administrated in double stimulation protocol (1475.31 ± 860.42 vs. 2605.21 ± 899.16, $p < 0.001$). The rate of two pronucleus (85.32% vs. 60.50%, $p < 0.001$) and formation of high-grade cleavage-stage embryos (45.52% vs. 34.75%, $p = 0.002$) were higher in double stimulation protocol. Ultimately, both the

calculated clinical pregnancy rate (28.87% vs. 19.81%, $p = 0.143$) and embryo implantation rate (20.69% vs. 14.55%, $p = 0.156$) were higher in double stimulation protocol in tendency, but without statistical difference.
Limitations, reasons for caution: It was a single-center study with small sample size.
Wider implications of the findings: Double stimulation protocol will become the first-line protocol for the ovarian stimulation in POR by the virtue of safer, lower-cost, and less side-effects.
Trial registration number: ChiCTR-IOR-14005521.

P-663 Hydrogen sulfide is an endogenous modulator of vaginal lubrication

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Study question: Our objective was to study the role(s) of H_2S in vaginal lubrication and to determine the mechanisms underlying it.
Summary answer: CSE/H_2S pathway participates in the regulation of vaginal lubrication, which is mediated by vaginal epithelial K^+ and Cl^- ion transport.
What is known already: Vaginal dryness is a common problem for women during and after menopause, which may result from inadequate vaginal lubrication. Vaginal fluid derived from transudate, glandular secretion and epithelial ion transport contributes to the vaginal lubrication. H_2S is not only a prosecretory gasotransmitter which regulates ion transport in colon, duodena and lung, but also play an important role in oocyte maturation, preeclampsia and smooth muscle relaxation in uterine and vagina. However, the role of H_2S in vaginal lubrication is not extensively studied.
Study design, size, duration: For *in vitro* experiments, isolated rat vaginal tissues were homogenized or treated with exogenous H_2S donor, NaHS with or without inhibitors and ion substitution, while vaginal canals were treated by NaHS in the presence or absence of inhibitors in anesthetized rats.
Participants/materials, setting, methods: The expression of enzyme, the production of H_2S, and the effect of H_2S on vaginal epithelial ion transport and fluid secretion was studied in rats by RT-PCR/western blot analysis, immunohistochemistry, H_2S synthesizing activity assay, short circuit current, vaginal lubrication measurement and ion chromatography.
Main results and the role of chance: Cystathionine γ-lyase (CSE) mRNA and protein were predominantly expressed in rat vaginal epithelium. NaHS caused concentration-dependent changes in I_{SC} across rat vaginal epithelium, which composed of an initial decrease phase and a following increase phase. Cl^- substitution and cystic fibrosis transmembrane conductance regulator (CFTR) inhibitor siginificantly abolished the increase phase in I_{SC}, suggesting this phase is Cl^- and CFTR dependent ($P < 0.05$), while the decrease phase was sensitive to ATP-sensitive K^+ (K_{ATP}) channels blocker ($P < 0.05$). In addition, NaHS significantly enhanced vaginal lubrication in the anesthetized rats ($P < 0.05$), of which the effect is prevented by CFTR and K_{ATP} channels inhibitors ($P < 0.05$). In addition, the ionic concentration of K^+ and Cl^- in rat vaginal fluid was significantly increased by NaHS treatment ($P < 0.05$).
Limitations, reasons for caution: Further studies on vaginal lubrication under the conditions of estrogen deprivation, a preclinical model of menopause, are urged to better delineate the role of endogenous H_2S in it.
Wider implications of the findings: The physiopharmacological effects of H_2S may provide an alternative, effective and potential therapy to alleviate vaginal dryness or inadequate lubrication in women during and after menopause by the application of exogenous H_2S donor to female vagina.
Trial registration number: Not applicable.

P-664 Low intensity laser therapy: possible strategy to restore fertility in premature ovarian failure induced by chemotherapy?

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Study question: Premature ovarian failure (POF) is characterized by the disappearance of ovarian follicles in young women, which may be caused by chemotherapy.
Summary answer: Therefore, the biomodulator effect of LLLT improves follicular dynamics in the early stages and thus preserves ovarian reserve in the POF model induced with CTX.
What is known already: POF treatments, which consist mainly of hormone therapies, are not completely effective. The present work proposes photobiomodulation as a strategy to protect the ovary and restore fertility in cancer patients undergoing chemotherapy by local application of low level laser therapy (LLLT).
Study design, size, duration: Experimental animal model and basic science.
Participants/materials, setting, methods: The objectives were: (a) To evaluate the *in vivo* effect of LLLT in adult mice ovaries on follicular dynamics, (b) To analyze the *in vitro* effect of LLLT in a culture of rat granulosa on cell proliferation and expression of VEGF, (c) To evaluate the *in vivo* effect of LLLT in the POF model induced by chemotherapy on ovarian folliculogenesis and expression of Anti-Müllerian Hormone (AMH).
Main results and the role of chance: For objective a, F1 mice (BalbC × C57/BL6) (8 weeks) were used and LLLT (2, 4 and 8 J/cm^2) was applied. For objective b, a culture of rat granulosa cells (GC) (Sprague–Dawley, 21–23 days) was performed and LLLT (1–10 J/cm^2) was applied. For the purpose c, POF model was induced with cyclophosphamide (CTX) in F1 mice and LLLT (8 J/cm^2/ovary) was applied.
The results showed that in adult mice the LLLT (4 and 8 J) increased ovarian reserve compared to control ($p < 0.01$). LLLT (8 J) induced cell proliferation and expression of VEGF 121 isoform in CG compared to untreated group ($p < 0.05$). LLLT (8 J) increased the % of primary, primary and preantral follicles, and decreased the % of atretic follicles in the POF model compared to the untreated group ($p < 0.05$). Besides, LLLT increased the ovarian expression of AMH in POF model compared to the untreated group.
Limitations, reasons for caution: The investigation needs more time.
Wider implications of the findings: Therefore, the biomodulator effect of LLLT improves follicular dynamics in the early stages and thus preserves ovarian reserve in the POF model induced with CTX.
Trial registration number: No.

P-665 Cardiovascular risk profile of women beyond 45 years of age previously diagnosed with premature ovarian insufficiency compared to premenopausal women of comparable age

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Study question: What are the differences in cardiovascular risk profile characteristics between women with premature ovarian insufficiency (POI) and premenopausal women of comparable age?
Summary answer: Women with POI exhibited few increased cardiovascular risk factors, and no signs of increased subclinical atherosclerosis compared to premenopausal controls of comparable age.
What is known already: Cardiovascular diseases (CVD) represent the world's leading cause of death amongst women. A young age at menopause has been associated with increased CVD.
Study design, size, duration: Cross-sectional case control study conducted within the University Medical Center Utrecht and Erasmus MC, University

Medical Center Rotterdam, the Netherlands, including women above 45 years of age who were previously diagnosed with POI ($n = 83$), and premenopausal controls from the general population of comparable age ($n = 266$).

Participants/materials, setting, methods: The outcome measures consisted of:

blood pressure, body mass index, waist circumference, electrocardiogram (ECG), bilateral carotid intima media thickness (C-IMT), estradiol, testosterone, androstenedione, dehydroepiandrosterone sulfate (DHEAS), sex hormone binding globulin (SHBG), insulin, glucose, lipids, thyroid stimulating hormone (TSH), freeT4, NTpro-BNP, C-reactive protein (CRP), uric acid, creatinine, homocysteine. Potential associations between POI status and cardiometabolic features, mean C-IMT and atherosclerotic plaque presence were assessed.

Main results and the role of chance: Women with POI exhibited an increased waist circumference (β = 5.7; 95% CI: 1.6, 9.9, $P = 0.007$), CRP (β = 0.75; 95% CI: 0.43, 1.08, $P < 0.001$) and free-T4 levels (β = 1.5; 95% CI: 0.6, 2.4, $P = 0.001$), and lower NTpro-BNP (β = –0.35; 95% CI: –0.62, –0.08, $P = 0.011$), estradiol (β = –1.98; 95% CI: –2.48, –1.48, $P < 0.001$), testosterone (β = –0.21; 95% CI: –0.37, –0.06, $P = 0.008$) and androstenedione (β = –0.54; 95% CI: –0.71, –0.38, $P < 0.001$) concentrations compared to controls, after adjusting for confounders. After adjustment, a trend towards increased hypertension (OR = 2.1; 95% CIs: 0.99; 4.56, $P = –0.05$) and decreased kidney function was observed in women with POI (creatinine β = 3.5; 95% CI: –0.05, 7.1, $P = 0.05$, GFR β = –3.5; 95% CI: –7.5, 0.46, $P = 0.08$). In addition, women with POI exhibited a lower mean C-IMT (β = –0.17; 95% CI: –0.21, –0.13, $P < 0.001$), and decreased odds of plaque presence compared to controls (OR = 0.08, 95% CI: 0.03; 0.26, $P = 0.006$).

Limitations, reasons for caution: The observed low adverse cardiometabolic profile in women with POI could be influenced by the inclusion of a relatively healthy POI population. Included women with POI were higher educated ($P < 0.001$) and had a lower BMI ($P = 0.001$), compared to controls from the general population.

Wider implications of the findings: Previous meta-analyses reported a modestly increased risk of ischemic heart disease in women with POI. Additional studies focusing on subclinical or manifest CVD at a later age are required to identify specific determinants of CVD risk in women with POI, in order to improve individualized counseling.

Trial registration number: www.clinicaltrials.gov, registration number NCT02616510.

P-666 Pharmacological inhibition of m-TORC1 prevents cyclophosphamide-induced over-activation of the primordial follicle pool through PI3K/AKT/m-TOR signaling pathway in mice

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Study question: We investigated the protective effects of pharmacological inhibition m-TOR rapamycin against chemotherapeutic-induced ovarian toxicity in a rat model.

Summary answer: Our results provide a rationale for exploring the possible use of rapamycin as a drug for the preservation of chemotherapy induced premature ovarian failure (POF).

What is known already: A new emerging hypothesis proposes that increased activation of follicles from the resting pool after chemotherapy and the eventual premature "burnout" of the primordial follicle reserve can cause POF. Several environmental chemicals that cause POF in rodent models have been shown to enhance PI3K and mTORC1 signaling pathways and lead to over-activation of primordial follicles. And one study has shown that rapamycin treatment can prevent the complete loss of primordial follicles that would otherwise occur in mice with an oocyte-specific deletion of PTEN.

Study design, size, duration: Adult female mice aged 8 weeks ($n = 52$) housed under specific pathogen-free conditions were treated with varying doses of Cy, ranging from single doses of 75 to 150 mg/kg to four weekly doses of 75 mg/kg, and ovaries were collected at time points of 1, 3, or 7 days after final treatment. Others ($n = 66$) were treated with rapamycin (5 mg/kg) every day, beginning 1 week before Cy treatment and ending 1 week after treatment.

Participants/materials, setting, methods: BALB/c females aged 8 weeks were used. H&E staining was used for differential follicle counts and proliferation staining was conducted with Ki67 antibody. Staining for apoptosis with the *In Situ* Cell Death Detection Kit, POD. Immunohistochemical and immunofluorescence staining conducted to show the localization of these proteins. Protein analysis of whole ovaries was conducted to assess changes in phosphorylation of the key activation proteins. Plasma AMH concentration was quantified with an AMH ELISA kit.

Main results and the role of chance: Quantification of the different follicle populations in the ovaries 1 week after Cy administration showed that at all doses, dormant (primordial) follicles suffered a proportionally greater reduction than did early growing follicles. This difference is apparent in a comparison of the ratio of early growing vs. dormant follicles. Protein analysis of whole ovaries from 8-week-old mice removed 24 h after Cy or PBS treatment was conducted to assess changes in phosphorylation of the key activation proteins AKT, m-TOR, and rpS6. Western blotting showed an increase in the phosphorylated forms of each of these molecules 24 h after Cy treatment. Immunohistochemical and immunofluorescence staining conducted on ovaries confirmed the localization of these proteins within the cytoplasm of the oocytes and granulosa cells of activated small follicles. Coadministration of the specific mTORC1 inhibitor rapamycin reduced follicle activation, thereby increasing follicle reserve and reduced phosphorylation of the key activation proteins AKT, m-TOR, and rpS6. AMH concentration was significantly reduced with Cy four weekly doses of 75 mg/kg ($P < 0.01$), but with coadministration of rapamycin, the concentration of AMH remained normal. Rapamycin alone did not alter the concentration of serum AMH.

Limitations, reasons for caution: Protein analysis conducted on whole-ovary lysates limits our ability to localize protein changes to specific cell types. Chronic treatment with m-TOR inhibitors has been reported to increase the risk of menstrual-cycle disturbances and ovarian cysts in women. Thus, further studies are required to evaluate such possible adverse effects of rapamycin.

Wider implications of the findings: The implication is that rapamycin may be clinically useful in maintaining at least a portion of the primordial follicle pool in the ovaries of women who are otherwise at risk for POF. This is especially relevant for women who are being treated with ovotoxic chemotherapeutic agents.

Trial registration number: no.

P-667 Unravelling the role of extra- and intra-ovarian androgen receptor-mediated mechanisms driving the development of ovarian polycystic ovary syndrome (PCOS) traits

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Study question: Does PCOS originate by a disruption in extra- or intra-ovarian mechanisms?

Summary answer: Our findings implicate predominantly an extra-ovarian origin for PCOS traits.

What is known already: PCOS is a common endocrine disorder causing anovulation and infertility, and is associated with an increase in metabolic disturbances. Hyperandrogenism is a key and most consistent feature of PCOS, but the role of androgen actions *via* the androgen receptor (AR) in its development is unclear. Previously we proved that the global loss of AR in AR knockout mice (ARKO) protects females from the induction of PCOS features, providing strong evidence for AR actions in the etiology of PCOS. However, the site of these AR actions remains to be defined.

Study design, size, duration: PCOS was induced by subdermal DHT implant for 3 months in control, granulosa cell specific-ARKO, neuron specific-ARKO; and control and global ARKO females that have undergone reciprocal ovary transplants.

Participants/materials, setting, methods: After 3 months of DHT exposure oestrous cycles were assessed and ovaries collected for assessment of ovarian PCOS features.

Main results and the role of chance: Compared to blank treated controls, DHT-induced PCOS control mice exhibited acyclicity, as did DHT-induced PCOS neuron-ARKO females ($P < 0.01$ for both). Treatment of GCARKO mice with DHT did not cause complete acyclicity, but instead mice displayed irregular cycles (blank 2.7 ± 0.2 vs. DHT 1.0 ± 0.2 cycles in 2 weeks, $P < 0.01$). Anovulation was assessed by enumerating corpora lutea (CL). DHT treatment

significantly reduced CL numbers in control (blank 9.7 ± 3.0 vs. DHT 0.8 ± 0.8, $P < 0.01$) and GCARKO females (blank 6.0 ± 0.6 vs. DHT 1.0 ± 1.0, $P < 0.01$). However, the loss of neuron AR signalling protected against the effect of DHT, with neuron-ARKO females exhibiting no significant loss in CL numbers (blank 7.2 ± 1.3 vs. DHT 5.0 ± 1.4). This finding infers that extra-ovarian (neuroendocrine) AR actions are the origin for ovulation disruption observed in PCOS. In support of this, reciprocal ovarian transplants between control ($AR^{+/+}$) and ARKO females revealed that DHT treatment induced acyclicity in control females implanted with control or ARKO ovaries, while the transplantation of control ovaries into ARKO recipients protected against the disruption of oestrous cycles by DHT.

Limitations, reasons for caution: This study carried out in rodents may not be directly extrapolated into humans.

Wider implications of the findings: By determining the role and contribution of intra-and extra-ovarian AR-mediated mechanisms in the development of PCOS traits, this better defines the origins of PCOS which may define improved therapeutic targets for novel and mechanism-based treatments.

Trial registration number: NA.

P-668 The controlled ovarian stimulation outcome is affected by Anti-Müllerian Hormone Receptor II genotypes

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Study question: To investigate whether *AMHRII 10A>G*, *1749C>T* and *-482A>G* genetic variants influence the ovarian response of women undergoing standard gonadotropin stimulation for *in vitro* fertilization (IVF/ICSI).

Summary answer: *AMHRII 1749C>T* and *-482A>G* genetic variants were associated with the follicular recruitment and growth of women undergoing controlled ovarian stimulation for IVF or ICSI.

What is known already: Anti-Müllerian hormone (AMH) plays a major role on tissue growth and differentiation. AMH, which is produced by the ovarian granulosa cells of pre-antral and antral follicles as early as the 36th week of gestation, limits the formation of primary follicles by inhibiting excessive follicular recruitment by FSH. AMH constitutes a reliable marker of ovarian dysfunction, of granulosa cell tumours as well as of ovarian reserve in ageing women and in patients undergoing IVF/ICSI. AMH exerts its biological effect through two receptors, AMHRI and AMHRII. AMHRII is expressed by Müllerian ducts and gonads, showing its primary role in reproduction.

Study design, size, duration: Three hundred Greek Caucasian women, with tubal or male-factor infertility, undergoing IVF or ICSI in a period of 2 years constituted the study population. Furthermore, 300 women with at least one spontaneous pregnancy participated in this prospective study as the control group. A detailed medical history was obtained from all women, while a physical examination was performed. All subjects, aged 28–38 years, had normal body mass index, normal menstrual cycles and no signs of hyperandrogenism.

Participants/materials, setting, methods: The FSH, LH, AMH and E_2 levels were determined at the third day of the menstrual cycle. The follicular size, the follicle and oocyte numbers were recorded during oocyte retrieval. All embryo transfers were performed at the third day of each cycle. Pregnancy was assessed 2 weeks after embryo transfer by β-hCG quantification. Clinical pregnancy was confirmed by observing fetal cardiac activity. *AMHRII 10AG* (rs11170555), *1749C>T* (rs2071558) and *-482A>G* (rs2002555) genetic variants were genotyped.

Main results and the role of chance: Significant differences in the follicle number and size were observed among *AMHRII* genotypes. Specifically, *1749CT* women presented with higher total follicle numbers compared to *1749CC* women (16.45 ± 10.21 vs. 13.45 ± 6.89, $p = 0.04$). Furthermore, *1749CT* genotype was associated with higher numbers of small follicles comparing with *1749CC* genotype (8.31 ± 8.01 vs. 5.46 ± 3.68, $p = 0.01$). Finally, *1749CT* women were characterized by lower large follicle numbers and higher oocyte numbers than *1749CC* women (5.87 ± 3.68 vs. 6.55 ± 4.17, $p > 0.05$ and 10.16 ± 5.69 vs. 8.62 ± 4.90, $p > 0.05$, respectively). However, no statistical significance was observed in these associations.

Regarding *AMHRII -482A>G* polymorphism, *-482AG* women tended to have higher total follicle numbers compared to *-482AA* women (16.50 ± 10.86 vs. 13.67 ± 6.85, $p = 0.07$). An association of *-482AG* genotype with higher numbers of small follicles comparing with the *-482AA* genotype was also observed (9.00 ± 8.13 vs. 5.48 ± 3.75, $p = 0.004$). In addition, *-482AG* women were characterized by slightly lower large follicle numbers and higher FSH levels compared to *-482AA* women (5.69 ± 4.30 vs. 6.53 ± 4.08, $p > 0.05$ and 7.58 ± 2.45 mIU/ml vs. 6.37 ± 2.2 mIU/ml, $p < 0.05$, respectively).

The *AMHRII 10A>G* genetic variant analysis showed no association with the hormonal profile and the COS outcome. Finally, no significant associations were observed between the *AMHRII* genotypes and the serum AMH levels or the clinical pregnancy rates.

Limitations, reasons for caution: Our study population was limited in Greek Caucasian women. Further studies in other ethnic groups and multicenter populations are needed to confirm our preliminary findings.

Wider implications of the findings: The current study pointed out the significance of *AMHRII* genotypes for the follicular recruitment and development. *AMHRII 1749C>T* and *-482A>G* genotype analysis could help in the tailoring of the COS protocols in order to achieve synchronization in the follicular growth and subsequently higher numbers of mature oocytes.

Trial registration number: N/A.

P-669 Acetyl-L-Carnitine (ALC) and L-Carnitine (LC) effects on neuroendocrine control of reproductive axis in hypogonadotropic women with functional hypothalamic amenorrhea (FHA)

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Study question: To evaluate the efficacy of the combined administration of Acetyl-L-Carnitine and L-Carnitine on gonadotropins secretion in patients with functional hypothalamic amenorrhea.

Summary answer: Our data show a positive effect of ALC and LC administration on the stress-induced abnormalities in patients affected by functional hypothalamic amenorrhea.

What is known already: Functional hypothalamic amenorrhea (FHA) is characterized by neuroendocrine impairment that negatively modulates endocrine function, mainly within the reproductive axis. FHA typically shows normal or hypo LH and hypoestrogenism. Up to now, a definite therapeutic strategy has not yet been found. Up to now stressors removal and the administration of naltrexone cloridrate, estriol have been reported to counteract the neuroendocrine impairment of FHA.

Study design, size, duration: A group of 24 patients with FHA were enrolled after informed consent and were administered ALC (250 mg/day) and LC (500 mg/day) for 12 weeks.

Participants/materials, setting, methods: Patients with FHA were subdivided in 2 groups according to LH plasma levels: Group A, hypo LH (LH < 3 mIU/ml) ($n = 14$) and Group B, normo LH (LH > 3 mIU/ml), ($n = 10$). All patients underwent to: baseline hormonal assessment, pulsatility test (for LH and FSH), GnRH stimulation test (bolus of 10 μg) (for LH and FSH) both before and at the 12th week of integrative treatment.

Main results and the role of chance: Under ALC + LC integrative treatment hypo LH patients showed a significant increase in LH plasma levels (from 1.7 ± 0.3 to 3.1 ± 0.5, $p < 0.01$) and in LH pulse amplitude ($p < 0.05$). Maximal LH response and AUC under GnRH stimulation were significantly increased in hypo LH patients ($p < 0.05$). Amylase and cortisol plasma levels were significantly decreased while insulin plasma levels were significantly increased ($p < 0.05$) only in hypo-LH patients. No changes were observed in the normo LH Group.

Limitations, reasons for caution: The only limit might be the number of the patients enrolled for this study

Wider implications of the findings: Our data are in agreement with previous reports on *in vitro* (pituitary cells) and *in vivo* (rat model and humans)

concerning the efficacy of ALC or LC on the stress-induced abnormalities of gonadotropin secretion. These are the first data on the combined administration of ALC + LC in stres-induced FHA
Trial registration number: Ethical Commitee no. 181/2012.

P-670 The impact of thyroid autoimmunity on IVF/ICSI outcome: a systematic review and meta-analysis

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Study question: Does thyroid autoimmunity (TAI) per sé impact on the outcome of *in vitro* fertilization (IVF)/intracytoplasmic sperm injection (ICSI) cycles?
Summary answer: Thyroid autoimmunity seems to have a detrimental impact on the course of pregnancy achieved through IVF/ICSI but not on the main outcomes of IVF/ICSI treatment.
What is known already: Thyroid autoimmunity is the most frequent autoimmune condition and the first cause of thyroid dysfunction among women of reproductive age. Notably, it has been associated with adverse obstetric outcomes during all trimesters of pregnancy. Furthermore, since most studies showed an increased prevalence of TAI among women attending infertility clinics, a detrimental impact of this condition on natural fertility and on the rate of success of assisted reproductive techniques (ART) has been suggested. However, to date, results are inconsistent.
Study design, size, duration: A systematic literature review and meta-analysis were conducted. A Medline search was performed to identify studies published from 1990 to 2015 on IVF/ICSI outcome in women with and without TAI. The primary outcome was delivery rate (DR). Our secondary outcomes were number of oocytes retrieved (NOR), fertilization rate (FR), implantation rate (IR), clinical pregnancy rate (CPR) and miscarriage rate (MR). We also extracted data on age and basal serum concentrations of thyroid stimulating hormone (TSH).
Participants/materials, setting, methods: Studies were excluded if: (i) only women with positive TAI were described without a comparison group of women without TAI, (ii) women were known to have hypothyroidism or hyperthyroidism or were receiving any treatment for thyroid dysfunction, (iii) serum TSH was not evaluated before starting the IVF/ICSI cycle, (iv) the participants were involved with donor oocytes treatment, (vi) each women performed more than one IVF/ICSI cycle. The study was conducted according to the PRISMA guidelines.
Main results and the role of chance: We selected 12 studies for the meta-analysis. Six of the included studies were prospective cohort studies and six were retrospective cohort studies. Compared with women with negative TAI, women with positive TAI had a lower DR (OR 0.65; 95% CI [0.49, 0.87]; $p = 0.004$; 9 studies; 4396 women; $I^2 = 66\%$); a higher MR (OR 1.44; 95% CI [1.06, 1.95]; $p = 0.02$; 12 studies; 4876 women; $I^2 = 35\%$) and a similar CPR (OR 0.90; 95% CI [0.77, 1.06]; $p = 0.22$; 12 studies; 4876 women; $I^2 = 7\%$), NOR (SMD 0.10; 95% CI [–0.09, 0.29]; $p = 0.28$; 5 studies; 1506 women; $I^2 = 47\%$), FR (OR 1.11; 95% CI [0.97, 1.27]; $p = 0.13$; 3 studies; 1082 women; $I^2 = 0\%$) and IR (OR 0.98; 95% CI [0.73, 1.32]; $p = 0.91$; 2 studies; 918 women; $I^2 = 0\%$). Both mean age (SMD 0.96; 95% CI [0.66, 1.27]; $p < 0.00001$; 9 studies; 3256 women; $I^2 = 85\%$) and serum TSH (SMD 0.24; 95% CI [0.15, 0.34]; $p < 0.00001$; 6 studies; 2098 women; $I^2 = 59\%$) resulted higher in women with TAI.
Limitations, reasons for caution: In the process of systematic review and meta-analysis the inferences assumed by the data are subject to the limitations of the primary studies. Furthermore, giving the possible confounding effects of age and serum TSH, further evidence is warranted prior to draw inferences on causality.
Wider implications of the findings: Thyroid autoantibodies do not impact on IVF/ICSI outcome in terms of number of oocytes retrieved and likelihood of fertilization, implantation and clinical pregnancy. On the contrary, TAI may have a detrimental effect on the course of pregnancy determining an increased risk of miscarriage and a decreased chance of delivery.
Trial registration number: Not applicable.

P-671 Majority of young females with occult premature ovarian insufficiency menstruate regularly: why we should not rely on menstrual status as a marker of ovarian reserve

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Study question: Is there any characteristic menstrual history that heralds the onset of occult premature ovarian insufficiency (POI) in young females?
Summary answer: No. Majority of the young females with occult POI (AMH ≤ 1 ng/mL) continue to menstruate regularly and report no menstrual abnormality in the last year.
What is known already: Diminished ovarian reserve or occult premature ovarian insufficiency (POI) may develop spontaneously and insidiously in young females. Even though its exact prevalance is unknown, it is believed that one in every 250 women <35 is diagnosed with occult POI. We aimed in this study to determine (1) the prevalence of previously undiagnosed occult POI in a specific population of young females using serum AMH level and (2) the percentage of these patients that have menstrual irregularities or other symptoms related to POI.
Study design, size, duration: Population based prevalence study in young female college students.
Participants/materials, setting, methods: A total of 1013 young female college students voluntarily participated in this study. The participants were asked to fill a questionnaire regarding the status of their menstrual cycles, menstrual symptoms, life style, exercise, contraceptive use, personal and family history of endocrine and metabolic diseases, and premature ovarian failure (POF). A blood sample was obtained during the first visit and serum AMH was measured with ELISA method using a commercially available kit.
Main results and the role of chance: Overall the mean ± SD for age and AMH of the patients were 20.3 ± 2.7 years and 3.2 ± 0.9 ng/mL, respectively. There was a strong negative association between age and AMH shown by correlation ($r = –0.134$, $p < 0.001$) and linear regression ($R^2 = –0.12$ $p < 0.001$) analyses. Thirty-five (3.4%) of 1013 students were diagnosed with occult POI as they had serum AMH levels ≤1 ng/mL (mean: 0.81 ± 0.2, range: 0.3–1). The mean age of these women was 24.5 ± 1.4 years. Thirty (85.7%) of them reported no menstrual irregularity in the previous year. The remaining five (14.3%) had at least one skipped menses in the last 6 months. 28 (80%) cases with POI denied any family history of premature ovarian failure in their mothers or other first degree relatives. All cases with POI had their diagnoses confirmed by early follicular elevated FSH (22 ± 2.5 mIU/mL) and lower antral follicle counts (2.6 ± 0.4) and were advised for additional tests and fertility preservation options during a second visit.
Limitations, reasons for caution: It is unclear whether these findings can be extrapolated to a larger population. AMH levels were measured only once in these patients. Serial measurements are not available. Long term follow-up is not available and thus fertility outcome and the timing of menopause cannot be assessed.
Wider implications of the findings: This study emphasizes that menstrual status is not a reliable marker of ovarian reserve and young females with critically diminished ovarian reserve may continue to menstruate regularly without any characteristic menstrual abnormality heralding POI. It also underscores the importance of screening young females for possible occult POI.
Trial registration number: None.

P-672 Anovulatory patients demonstrate a sharp drop in LH levels in response to GnRH antagonist administration in IVF cycles

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Study question: Are anovulatory patients hyper-responsive to GnRH antagonist administration as compared to normal cycling patients undergoing IVF treatment?
Summary answer: The drop in LH concentration from stimulation start to that observed before ovulation triggering was almost two fold greater in anovulatory vs. ovulatory patients.

What is known already: The ideal ovarian stimulation protocol is under constant debate. The need for threshold LH levels during ovarian stimulation is clearly demonstrated in hypogonadotropic hypogonadic patients, as well as in cycling patients treated with high doses of gonadotropin-releasing hormone (GnRH) antagonists. The Ganirelix dose-finding study showed very low implantation rates in the high-dose groups (1 mg, 2 mg). In that study, the stimulation dynamics in LH suppressed subjects was remarkable for very low estradiol levels. Importantly, we have recently found that 25% of patients receiving only 0.25 mg of GnRH antagonists displayed "over-suppression" and may benefit from LH supplementation.
Study design, size, duration: A retrospective study analyzing IVF cycles (n = 305) at a tertiary center IVF unit. Patients were (n = 190) or were not (n = 115) scheduled by estradiol 2 mg bid before gonadotropin stimulation. GnRH-antagonist (0.25 mg) was administered once a lead follicle of 14 mm was observed. The response to GnRH-antagonist was calculated as the percent change in LH concentration from stimulation start to that observed before ovulation triggering. This result was compared between ovulatory (n = 270) and anovulatory patients (n = 35).
Participants/materials, setting, methods: In this study, we included all consecutive IVF cycles during the year 2015, at Rambam Hospital IVF unit, in which downregulation with GnRH antagonist (Cetrotide 0.25 mg) was used. Excluded were cycles with pituitary down regulation protocols not using GnRH antagonist, concurrent medical conditions that may interfere with pregnancy outcome, and cycles where all embryos were frozen. The primary endpoint was the percentage of drop in LH levels following antagonist administration.
Main results and the role of chance: Anovulatory patients were younger (28.3 ± 5.6 vs. 34.3 ± 6.5 years; $p < 0.001$), with higher BMI (27.3 ± 6.0 vs. 24.5 ± 4.9; $p = 0.002$) and demonstrated higher ovarian reserve parameters (AFC: 19.9 ± 10.3 vs. 9.9 ± 5.5; $p < 0.001$, basal FSH: 5.3 ± 1.5 vs. 7.2 ± 2.8 IU/L; $p < 0.001$) compared to ovulatory patients. The drop in LH concentration from stimulation start to that observed before ovulation triggering was almost two fold greater in anovulatory vs. ovulatory patients (66.4 ± 26.3 vs. 38.9 ± 65.0 percent; $p = 0.01$). Number of oocytes (8.7 ± 5.8 vs. 7.7 ± 5.6) fertilizations (4.7 ± 4.3 vs. 4.3 ± 3.6), cleavage stage embryos (3.2 ± 3.1 vs. 2.8 ± 2.2) and transferred embryos (1.5 ± 0.8 vs. 1.7 ± 0.8) were similar (p = NS). Implantation rates (27.8 ± 42.2 vs. 11.5 ± 28.6%; $p = 0.003$) were higher in anovulatory vs. ovulatory patients.

Cycle scheduling with estradiol pretreatment increased LH levels on the day of stimulation start (6.7 ± 4.5 vs. 5.6 ± 3.4 IU/L; $p = 0.03$) however the drop in LH concentration from stimulation start to that observed before ovulation triggering was similar (42.8 ± 52.1 vs. 42.9 ± 76.5%; p = NS). Number of oocytes (7.6 ± 5.4 vs. 8.1 ± 5.9) fertilizations (4.4 ± 3.6 vs. 4.3 ± 3.7), cleavage stage embryos (2.8 ± 2.3 vs. 2.9 ± 2.4) transferred embryos (1.6 ± 0.8 vs. 1.6 ± 0.8) and implantation rates (14.5 ± 32.3 vs. 11.8 ± 28.4%) were all similar (p = NS).
Limitations, reasons for caution: This is a descriptive retrospective study and therefore direct cause and effect relationships between the measured parameters cannot be fully established.
Wider implications of the findings: Over-suppressing endogenous LH levels leads to poor reproductive outcome. We now demonstrate that anovulatory patients are more susceptible to this detrimental effect of GnRH-antagonist administration. We suggest that in these patients LH supplementation may be beneficial. Moreover, estradiol based cycle scheduling does not affect pituitary response to GnRH antagonists.
Trial registration number: NA.

P-673 Clinical pregnancy and implantation rates decline gradually and insidiously with small rises in serum progesterone on the HCG day: an analysis of 3,767 IVF cycles

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Study question:
Is it possible that even small rises in serum P before ovulation trigger have subtle detrimental effects on the success of pregnancy in IVF cycles?
Summary answer:
Yes. The CPR and IR begin to decrease gradually and insidiously with small increments of serum P on the hCG day.
What is known already:
Serum P may prematurely rise before ovulation trigger during multifollicular development in IVF cycles and reduce the CPR and IR by impairing endometrial receptivity. But it is unclear whether small rises in serum P have also subtle deleterious effects on the success of pregnancy. It is also unknown if the relative contributions of AFC ≥ 14 mm, E2 on the HCG day and the number of eggs retrieved are the same in predicting serum P on the hCG day in stimulated IVF cycles. We aimed to address these issues in the current study.
Study design, size, duration:
A non-interventional, retrospective cohort data of a single center between 2006 and 2015.
Participants/materials, setting, methods:
3,767 IVF cycles with GnRH agonist long protocol were included. Of these IVF cycles 2,971 were fresh embryo transfer (ET) and the remaining 796 were frozen ET cycles. The patients were divided into 9 different groups based on their serum P level on the hCG day (<0.5/0.5–0.9/1–1.4/1.5–1.9/2–2.4/2.5–2.9/3–3.4/3.5–3.9/4–4.5 ng/mL). The primary outcome measure of the study was to analyze the individual impact of each of these P threshold intervals on the CPR and IR per ET.
Main results and the role of chance:
Serum P on the hCG day was significantly associated with the success of pregnancy (OR (95%CI): 0.74 (0.67–0.82), $p < 0.001$) in fresh ET cycles. Such an association was not found in frozen ET cycles (0.84 (0.75–1.31), $p > 0.05$). The CPR and IR began to decline gradually and insidiously with rising serum P, but were not significantly compromised up to 3.5 ng/mL in fresh ET cycles. The proportions of low responders (<6 oocytes) decreased and high-responders (>12 oocytes) increased along with rising serum P on the hCG day. There were no cases who produced <6 oocytes and had a serum $P \geq 3.5$ ng/mL. Starting from this level serum P significantly decreased the success of pregnancy in normo-responders (6–12 oocytes) (OR:0.32 (0.16–0.54), $p < 0.001$), but not in high-responders (OR:0.32, (0.27–1.44), $p = 0.36$). Serum P was significantly associated with AFC ≥ 14 mm on day 10th of the stimulation ($R2 = 0.68$ $p < 0.001$), E2 on the hCG day ($R2 = 0.72$ $p < 0.001$) and the numbers of total ($R2 = 0.45$ $p < 0.01$), and mature oocytes retrieved ($R2 = 0.38$, $p < 0.01$). Zero order, partial and part correlations, and multi-collinearity statistics with tolerance and variance inflation factor revealed that the relative contribution of AFC ≥ 14 mm is similar to E2 (hCG day) and oocyte number in predicting serum P on the hCG day.
Limitations, reasons for caution:
It is unclear why some patients have premature P elevations while the others do not despite both have similar IVF characteristics. Furthermore, AFC ≥ 14 mm, E2 on the hCG day and oocyte number can collectively explain only 70% of the variance in serum P with the remaining 30% belonging to unknown factors.
Wider implications of the findings:
Serum P on the hCG day is strongly associated with the magnitude of ovarian stimulation. Therefore, premature rise in serum P is likely to occur as a result of the inability of the ovary to handle high input precursor steroids generated during multifollicular development in stimulated IVF cycles.
Trial registration number:
None.

P-674 Analysis of the XIST gene C43G mutation in relation to X-chromosome inactivation patterns in patients with idiopathic primary ovarian insufficiency

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Study question: Is the X-inactive specific transcript (*XIST*) gene promoter mutation associated with idiopathic primary ovarian insufficiency (POI) which shows skewed X-chromosome inactivation (XCI)?
Summary answer: The *XIST* gene mutation was not associated with XCI patterns of POI, implying that the role in the pathogenesis of POI is not clear yet.

What is known already: Cryptic abnormalities of X-chromosome or gene mutations may lead to skewed XCI and result in POI. There were reports that incidence of skewed XCI was significantly higher in the POI group and a promoter C43G mutation of the *XIST* gene was associated with skewed XCI. Therefore, the *XIST* gene is an attractive candidate gene for underlying skewed XCI which has been documented in the structural abnormalities of X-chromosome and could have a role in the pathogenesis of idiopathic POI.
Study design, size, duration: Case–control study. The subjects consisted of 126 idiopathic POI patients (35.3 ± 13.9 years old, mean ± SD) and 126 healthy controls (35.2 ± 13.9 years) that had normal menstrual cycles. The women recruited as control were age-matched.
Participants/materials, setting, methods: The XCI status was evaluated by the methylation assay of androgen receptor locus in cases and controls. For the promoter C43G mutation in the *XIST* gene, genotyping was identified using polymerase chain reaction (PCR)-restriction fragment length polymorphism (RFLP) analysis. The incidence of skewed XCI and the *XIST* gene mutation in POI group were compared with those of control, and the *XIST* gene mutation was also evaluated in relation to XCI patterns in POI population.
Main results and the role of chance: The incidence of skewed XCI on all three levels (≥90%, ≥80% and ≥70%) was similar between POI patients and controls, and showed random pattern. Within POI population, the patterns of skewed XCI were not associated with age at the time of POI and LH, FSH, estradiol levels. There was no C43G mutation in the *XIST* gene, on the contrary to our expectation, in both idiopathic POI patients and controls. Result of no mutation was confirmed by sequence analysis. Therefore, the *XIST* gene promoter mutation was not associated with XCI patterns and also not idiopathic POI in a Korean population. Small deletions or mutations in X-linked genes do not appear to be a common feature of POI and X-linked genes involved in POI may be too few or not able to interfere with XCI.
Limitations, reasons for caution: Study is limited to a Korean population, so other studies from different populations will be helpful to replicate and confirm these results in the idiopathic POI.
Wider implications of the findings: Our findings suggest that genetic variants in X-inactivation pathway may not influence the susceptibility of idiopathic POI. This is the first report regarding the association between the *XIST* gene variations and XCI patterns in idiopathic POI.
Trial registration number: N/A.

P-675 The rate of ovarian failure in 12 months following adjuvant chemotherapy in breast cancer patients according to age group

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Study question: What is the rate of chemotherapy induced ovarian failure (CIOF) in 12 months following completion of chemotherapy in breast cancer patients in different age groups?
Summary answer: The CIOF rate in age groups 20–24, 25–29, 30–34, 35–39, and 40–44 were 0% (0/1), 0% (0/8), 3.8% (1/26), 12.9% (13/95), and 20.8% (35/168), respectively.
What is known already: In the previous studies that assessed the rate of CIOF in breast cancer, the overall rate was reported to be between 22 and 85.2%. The majority of studies did not assess the rate according to age group, and in one study, the rate in age group 35–45 years was as high as 95%.
Study design, size, duration: Retrospective study of women who underwent adjuvant chemotherapy between January 2009 and December 2012 in a tertiary medical center.
Participants/materials, setting, methods: This study included 296 women of age 20–44 who have agreed to have their serum follicle stimulating hormone (FSH) levels tested between 9 months and 15 months following completion of chemotherapy. Any concurrent use of gonadotropin releasing hormone agonists for fertility preservation were excluded.
Main results and the role of chance: The overall rate of CIOF was 16.2%. The rate of CIOF in age groups 20–24, 25–29, 30–34, 35–39, and 40–44 were 0% (0/1), 0% (0/8), 3.8% (1/26), 12.9% (13/95), and 20.8% (35/168), respectively. There was a significant increasing trend of CIOF with increasing age ($P = 0.006$). The CMF chemotherapy regimen showed a higher rate of CIOF compared to anthracycline and/or taxane containing chemotherapy regimen, but the difference was statistically not significant (36.4% and 15.4%, respectively, $P = 0.084$).
Limitations, reasons for caution: This was a retrospective study.
Wider implications of the findings: This is the largest study up to date that assessed the rate of CIOF diagnosed by serum FSH level. The rate of CIOF reported in our study is lower than that reported in other previous studies.
Trial registration number: Not applicable.

P-676 Recombinant FSH plus highly purified hMG versus recombinant FSH on ovarian response and clinical outcomes in long GnRH agonist protocol: A prospective, randomized, controlled trial

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Study question: Could the treatment of recombinant FSH (r-FSH) *plus* highly purified (HP-hMG) improve the ongoing pregnancy rate (PR) compared with r-FSH in long GnRH agonist protocol?
Summary answer: Non statistical differences were found in terms of ongoing pregnancy rate between r-FSH *plus* HP-HMG group and r-FSH group.
What is known already: The FSH isoform were different in r-FSH and hP-HMG, moreover hCG driven LH activity added to HP-hMG. There was no large randomized trial compared r-FSH with r-FSH *plus* HP-hMG on ovarian response and clinical outcomes in IVF/ICSI cycles.
Study design, size, duration: A single-centre RCT was performed between June 2014 and June 2015 with 579 women undergoing IVF/ICSI cycles. The patients were randomly assigned to one of the two groups,, by giving them a code number from a computer generated randomization list, in order of enrolment. The randomization was took place on the first day of ovarian stimulation.
Participants/materials, setting, methods: All the patients were less than 38 years old in their first cycle for IVF/ICSI with luteal phase long GnRH agonist protocol. Two hundred and seventy five patients were treated with r-FSH in combination with hP-HMG from the beginning of stimulation, and three hundred and four patients were treated with r-FSH. Depending on the allocation group, received either ovarian stimulation with 75~150 IU r-FSH + 75 IU hP-hMG or only 150~225 IU r-FSH.
Main results and the role of chance: The main outcome measures were ongoing PR and number of oocytes retrieved. Ongoing pregnancy rates were 126/211 (59.7%) in the r-FSH *plus* hP-hMG group vs. 105/188 (55.9%) in the r-FSH group [odds ratio (OR) 1.17, 95% CI 0.79–1.75, $P = 0.435$]. The number of oocytes retrieved was significant lower in the r-FSH *plus* HP-hMG group than r-FSH group (13.7 ± 6.1 vs. 15.1 ± 7.2, $P = 0.008$).
Limitations, reasons for caution: This was a open labeled study and all the patients were from a single IVF center.
Wider implications of the findings: Our results demonstrated that no differences in terms of ongoing PR between r-FSH *plus* HP-hMG group and r-FSH group, in long GnRH agonist protocol for IVF/ICSI cycles. R-FSH had the higher potency of follicular recruitment than r-FSH *plus* hP-HMG.
Trial registration number: ChiCTR-TRC-14004965.

P-677 The usage of Utrogestan and hMG protocol in patients with polycystic ovarian syndrome undergoing controlled ovarian hyperstimulation during IVF/ICSI treatments

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Study question: Could the delivery of progesterone soft capsules (Utrogestan) from the early-follicular phase inhabit the premature LH surges in polycystic ovarian syndrome (PCOS) women undergoing controlled ovarian hyperstimulation (COH) during IVF/ICSI treatments?
Summary answer: Consistent LH suppression was achieved along with improved pregnant outcomes following frozen-thawed embryo transfer (FET) in PCOS women undergoing COH with Utrogestan and hMG protocol.

What is known already: Although a variety of protocols are used for COH in PCOS women, the clinical outcomes still remains unsatisfactory. Our previous study showed that Utrogestan was feasible for preventing premature LH surges in normalovulatory women with component oocytes and embryos. So far, there were no publications reporting the usage of Utrogestan and hMG protocol in PCOS patients during IVF/ICSI treatments.
Study design, size, duration: 200 PCOS women were recruited from April 2013 to April 2015 in this retrospective study including 123 patients using Utrogestan and hMG protocol and 77 patients using short protocol.
Participants/materials, setting, methods: PCOS patients with 25–40 years old were eligible to participate. Utrogestan 200 mg/d and hMG 225 IU were administered from cycle day3. When the dominant follicles reached mature, GnRH-a 0.1 mg was used for trigger. The clinical results were compared in terms of the hormone profile, embryo results and pregnant outcomes.
Main results and the role of chance: No premature LH surges were detected in all the participants. No significant between-group differences were observed in the number of oocytes retrieved (13.27 ± 7.46 vs. 13.1 ± 7.98), the number of viable embyos (5.57 ± 3.27 vs. 5 ± 2.79), the mature oocyte rate (90.14% ± 11.81% vs. 93.02% ± 8.95%), and cleavage rate (97.69% ± 6.22% vs. 95.89% ± 9.57%). However, the fertilization rate (76.11% ± 19.04% vs. 69.34% ± 21.81%, $P < 0.05$), viable embryo rate per oocyte retrieved (39.85% vs. 34.68%, $P < 0.05$), biochemical pregnancy rate (71.72% vs. 56.67%, $P < 0.05$), clinical pregnancy rate (64.65% vs. 51.65%, $P < 0.05$), and implantation rate (46.46% vs. 31.35%, $P < 0.05$) in the Utrogestan and hMG protocol were significantly higher than that in the short protocol.
Limitations, reasons for caution: A major limitation of our study is the retrospective design of this study. In addition, the limited number of participants enrolled also decreased the power of this study.
Wider implications of the findings: Our findings provide new insights for the treatment of PCOS women undergoing COH in combination with FET. The optimization of this novel protocol remains to be explored to warrant the follicle development in an appropriate microfollicular milieu and improve the reproductive outcomes in PCOS patients.
Trial registration number: No trial registration number.

P-678 The expressions of ovarian steroidogenic enzymes do not increase proportionally after FSH, creating a shunting that promotes progesterone output in the granulosa cells without luteinization

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Study question:
If premature rise in serum P before ovulation may occur in stimulated IVF cycles can gonadotropin stimulation possibly play a role in this scenario?
Summary answer:
Yes. FSH alters the expression patterns of the enzymes involved in ovarian steroidogenesis that facilitates premature rise in serum P in a dose-dependent manner.
What is known already:
Serum progesterone may prematurely rise before ovulation trigger during multi-follicular development and reduce pregnancy rate in stimulated IVF cycles. The underlying molecular pathogenetic mechanism is unclear. We aimed in this study to explore if gonadotropin stimulation alters the expression of the ovarian steroidogenic enzymes (stAR, SCC, 3β-HSD, 17α-OH, 17β-HSD and aromatase) in a way that creates a relative shunting in steroidogenetic pathways leading to premature rise in serum P before ovulation.
Study design, size, duration:
A translational research study combining *in vivo* and *in vitro* models of human ovarian cortical samples and granulosa cells.
Participants/materials, setting, methods:
Ovarian cortical samples ($n = 10$) and non-luteinizing mitotic granulosa cells (HGrC1) were stimulated with rec-FSH at 12.5–25– 50 mIU/mL concentrations for 24 and 48 h. Then they were compared for the expressions of the steroidogenic enzymes at mRNA level by real-time quantitative qRT-PCR and protein level by western blot and ELISA.
Main results and the role of chance:
FSH significantly increased the expressions of its own receptor (4.22 fold, $p < 0.01$), stAR (1.82 fold, $p < 0.01$), SCC (2.19 fold, $p < 0.01$), 3β-HSD (2.63 fold, $p < 0.01$), and aromatase (5.19 fold, $p < 0.001$) in a dose-dependent manner when compared to their unstimulated controls at the same time point. By contrast, there were no meaningful changes in the expressions of 17α-OH and 17β-HSD after stimulation with FSH (ranging from 1.01 to 1.13). In line with FSH induced upregulation in the expressions of 3β-HSD and aromatase, *in vitro* P production from the samples increased along with E2. The mean levels at 24, 48, 72 and 96 h were 770 ± 45, 1222 ± 134, 1670 ± 167 and 1920 ± 245 pg/mL, respectively for E2; and 0.65 ± 0.1, 0.87 ± 0.2, 1.23 ± 0.4 and 2.13 ± 0.5 ng/mL, respectively for P. Such increases were not noted in unstimulated samples as their E2 (156 ± 22 to 255 ± 34 pg/mL) and P levels (0.2 to 0.4 ng/mL) were only slightly increased during the same culture period. Quantitative immunoblotting confirmed that protein levels of 3β-HSD and P were also increased after FSH stimulation, indicating that FSH induced upregulation in the transcription was accompanied by increased translation at protein level.
Limitations, reasons for caution:
It would be the most reliable and informative model to obtain granulosa cells of the growing antral follicles during the course of ovarian stimulation and compare the expression of these enzymes between the patients with and without premature progesterone rise before ovulation. Unfortunately this is not possible for ethical reasons.
Wider implications of the findings:
Disproportional increases in the expressions of these steroidogenic enzymes after FSH and high input precursor steroids generated during multifollicular development in stimulated IVF cycles may create a relative shunting in the ovarian steroidogenesis that diverts these precursors into progesterone pathway and causes premature rise in serum P before ovulation trigger.
Trial registration number: None.
StAR: Cholesterole uptake
SCC: Cholesterole → Pregnenolone
3β-HSD: Pregnenolone → Progesterone
17α-OH: Pregnenolone → 17-OH Pregnenolone; Progesterone → 17-OH Progesterone
17β-HSD: DHEAS → Androstenedione; Androstenedione → Testosterone
Aromatase: Androgens → Estrogens

P-679 The comparison of Utrogestan 100 mg or 200 mg per day used for controlled ovarian hyperstimulation in normalovulatory women: a prospective, randomized, controlled trial

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Study question: Was the delivery of progesterone soft capsules (Utrogestan) 100 mg per day from the early-follicular phase feasible to suppress the premature LH surge in normalovulatory women undergoing IVF/ICSI treatments?
Summary answer: Utrogestan 100 mg per day can effectively block the premature LH surge in normalovulatory women undergoing controlled ovarian hyperstimulation (COH).
What is known already: Our previous study shows that Utrogestan 200 mg per day is an effective oral alternative for preventing premature LH surges in normalovulatory women undergoing COH, with optimal pregnant outcomes in frozen-thawed embryo transfer (FET) cycles. However, the LH value was found to be excessively suppressed, leading to an increased hMG dose compared with the short protocol. There were no reports published investigating the appropriate dose of Utrogestan.
Study design, size, duration: 150 women were recruited from September 2014 to October 2015 in this prospective trial and participants were allocated randomly to the low dose group (Utrogestan100 mg per day)and the high dose group (Utrogestan 200 mg per day).
Participants/materials, setting, methods: Normal ovulatory patients with 25- to 40-year-old were enrolled. Utrogestan 100 mg or 200 mg per day and hMG 225 IU were administered from cycle day3. When the dominant follicles reached mature, GnRH-a 0.1 mg was used for trigger. The hormone profile,

embryo results and pregnant outcomes were analysed exhaustively to discriminate the differences between the two groups.
Main results and the role of chance: Consistent LH suppression was achieved during COH with Utrogestan 100 mg per day, and the number of patients with profound LH suppression (LH < 1.2 IU/L) in the low dose group was significantly less than that in the high dose group. The low dose group was characterized by a reduced hMG dose (1646 ± 223.95 IU vs.1747 ± 366.76 IU, $P > 0.05$), without statistic difference. The number of oocytes retrieved in the low dose group was comparable with that in the high dose group (9.87 ± 5.77 vs.10.25 ± 5.43, $P > 0.05$). No significant between-group differences were observed in the number of mature oocytes (8.24 ± 4.88 vs. 9.01 ± 4.87, $P > 0.05$), fertilized oocytes (6.73 ± 4.23 vs. 6.85 ± 4.19, $P > 0.05$), viable embryos (3.73 ± 2.34 vs. 4.15 ± 3.17, $P > 0.05$), the clinical pregnancy rate (50% vs. 51.32%, $P > 0.05$), or implantation rate (38.67% vs. 36.05%, $P > 0.05$).
Limitations, reasons for caution: A major limitation of our study is the limited number of participants enrolled which may decrease the power of this study.
Wider implications of the findings: Our finding confirmed Utrogestan 100 mg per day was able to prevent premature LH surges, and implied the profound LH suppression was correlated with the dose of Utrogestan, which may provide new insights for the optimization of this novel protocol by individuals.
Trial registration number: The trial was registered with the Chinese Clinical Trial Registry (ChiCTR-ONRC-14005277).

P-680 Oocyte developmental competence in women younger than 35 is independent from the ovarian reserve measured by antral follicular count

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Study question: Is higher ovarian reserve associated with higher oocyte developmental competence, measured as the ability to give rise to viable pregnancy following assisted reproduction (ART)?
Summary answer: Ovarian reserve is not associated with oocyte developmental competence following ART.
What is known already: Antral follicular count (AFC) is a good marker of ovarian reserve, but its relationship with oocyte developmental competence is debated. Women with higher AFC on average give rise to more oocytes and embryos, leading to a more efficient embryo selection, and higher pregnancy rates. Ideally, oocyte quality could be tested by separating oocyte yield from AFC, i.e., producing embryos from a pool of oocytes of the same size across different AFC. The oocyte donation model, where recipients are allocated a number of oocytes independent from donor AFC, allows testing the developmental competence of oocytes from women with differing AFC.
Study design, size, duration: Retrospective cohort study of 1985 oocyte donation cycles, corresponding to 3316 embryo transfers (ET) in recipients performed between January 2010 and May 2014. Each recipient received a number of MII ranging from 4 to 15, based on medical need and irrespectively of donor AFC. Oocyte donors were between 18 and 35 years old, and were stimulated using GnRH antagonist protocol triggered with GnRH-agonist. Recipient's endometrial preparation was performed with estrogens, administered either orally or transdermally.
Participants/materials, setting, methods: The effect of AFC on stimulation outcomes was assessed by linear regression. Effect of AFC on biochemical, clinical, ongoing, and live birth rates was assessed by logistic regression adjusting for age, BMI, phenotype, smoking habit, sperm origin (partner or donor), sperm status (fresh or frozen), number of embryos transferred, day of ET, and embryo quality score of the cohort produced.
Main results and the role of chance: The mean AFC in oocyte donors was 19.5, and SD 8.1, ranging from 4 to 60. Oocyte donors with higher AFC needed less total gonadotropin dose to reach trigger criteria (B −23.02, 95% CI [−25.72; −20.33], $p < 0.001$), and yielded a higher number of both cumulus-oocyte complexes (B 0.47, 95% CI [0.41, 0.53], $p < 0.001$) and MII oocytes (B 0.36, 95% CI [0.32, 0.41], $p < 0.001$). Oocyte donors with lower AFC had more cancellation of ovarian stimulation (OR 0.93, 95% CI [0.90, 0.96], $p < 0.001$), while donor's AFC did not affect either fertilization rate (B 0.001, 95% CI [0.000, 0.002] $p = 0.11$) or embryo quality score of the cohort of embryos produced (B -0.004, 95% CI [−0.009, 0.001] $p = 0.09$).

The number of MII oocytes assigned to each recipient, as well as the number of total embryos in cleavage stage for each recipient, were comparable across the range of donor AFC ($p = 0.09$ and $p = 0.08$, respectively). No association was found between AFC and biochemical pregnancy (OR 1.01, 95% CI [1.00, 1.02], $p = 0.20$), clinical pregnancy (OR 1.00, 95% CI [0.99, 1.01], $p = 0.63$), ongoing pregnancy (OR 1.00, 95% CI [0.99, 1.01], $p = 0.63$), and live birth (OR 1.00, 95% CI [0.99, 1.01], $p = 0.79$).
Limitations, reasons for caution: The main limitation of this study is its retrospective nature. Caution should be exerted when generalizing the results to infertile and/or older populations, where there is an age-related decline in oocyte quality, and to women <35 with an extremely low ovarian reserve (AFC <4).
Wider implications **of the findings:** The results suggest that, within the AFC range analyzed, the size of the ovarian reserve does not relate to the developmental competence of the oocytes in women younger than 35. This should be taken into account by gynecologists and first line physicians when prescribing, and interpreting, ovarian reserve tests.
Trial registration number: NA.

P-681 Amphiregulin mediates hCG-induced StAR expression and progesterone production in human granulosa cells

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Study question: To investigate the role of Amphiregulin (AREG) in human chorionic gonadotropin (hCG)-induced progesterone production and the underlying molecular mechanism in human granulosa cells.
Summary answer: AREG mediates hCG-induced StAR expression and progesterone production in primary human granulosa cells through binding Epidermal growth factor receptor (EGFR) and activating ERK1/2 signaling pathway.
What is known already: After ovulation, luteinizing hormone (LH) stimulates granulosa cells to produce progesterone, which plays a critical role in maintaining a successful pregnancy at the early embryonic stage. LH and/or hCG in granulosa cells rapidly induce AREG, a member of the epidermal growth factor (EGF) superfamily. However, whether AREG mediates LH/hCG-induced progesterone production remains unknown.
Study design, size, duration: Primary human granulosa cells were used as the study model to examine the involvement of AREG in hCG-induced StAR expression and progesterone production. Human serum and follicular fluid samples were obtained from 14 infertile women treated with *in vitro* fertilization (IVF) or intracytoplasmic sperm injection (ICSI).
Participants/materials, setting, methods: Real-time quantitative PCR (RT-qPCR) and western blot were used to measure mRNA and protein levels, respectively. The levels of AREG in human follicular fluid were measured using an enzyme-linked immunosorbent assay (ELISA). The progesterone levels in human follicular fluid and serum were measured using a electrochemiluminescence immunoassay (ECLIA).
Main results and the role of chance: Inhibition of EGFR and knockdown of AREG abolished hCG-induced StAR expression and progesterone production. Importantly, follicular fluid AREG levels were positively correlated with progesterone levels in follicular fluid and serum on oocyte-pick up (OPU) day and 2 days after OPU day. Treatment with AREG increased StAR expression and progesterone production. The stimulatory effects of AREG on StAR expression and progesterone production were abolished by inhibition of EGFR. Moreover, activation of ERK1/2, but not PI3K/Akt, signaling was required for AREG-induced upregulation of StAR expression and progesterone production.
Limitations, reasons for caution: Despite our study demonstrated that AREG mediates hCG-induced StAR expression and progesterone production in human granulosa cells, some other functions of AREG in the regulation of steroidogenesis remain undefined. In addition, future studies using a large clinical sample are needed to confirm the role of AREG in female reproduction.
Wider implications of the findings: To our knowledge, it is the first study revealing the physiological roles and molecular mechanisms of AREG in the regulation of StAR expression and progesterone production in human granulosa cells, which might help for developing new strategies for the treatment of clinical infertility.
Trial registration number: N/A.

P-682 Clinical outcomes of frozen–thawed embryo transfer in hyper responder patients who had final follicular maturation triggered by GNRH-agonist or hcg and all embryos cryopreserved

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Study question: Are clinical outcomes better in frozen–thawed embryos transfers when final follicular maturation in stimulation cycle were triggered by GnRH-agonist compared to hCG-trigger in hyper-responder patients?
Summary answer: Multiple regression analysis confirmed higher pregnancy chance in the following frozen–thawed embryo-transfer (ET) when GnRH-agonist for final follicular maturation is used, even adjusted for confounders.
What is known already: The use of GnRH-agonist for final oocyte maturation in clinical practice of IVF cycles has opened a new possibility to avoid ovarian hyperstimulation syndrome (OHSS) in hyper-responder patients. The administration of GnRH-agonist induces the release of gonadotropins that leads to LH surge, with the advantage of more physiological trigger and in a shorter extend compared to hCG. It has been reported that the use of GnRH-agonist trigger can prompt more MII oocytes in hyper-responder patients; on the other hand, a significant luteal phase defect may occur. The approach of freeze all embryos, following by frozen–thawed cycles can drive this issue.
Study design, size, duration: Retrospective observational study evaluating 127 frozen–thawed ICSI cycles between 2011 and 2015. Patients undergoing their first or second ovarian stimulation cycle, had at least 8 oocytes picked-up and didn't have embryos transferred in the fresh cycle. All embryos were cryopreserved and frozen–thawed transfers were placed in subsequent endometrial prepared cycle.
Participants/materials, setting, methods: Pituitary downregulation was obtained with GnRH-antagonist, and ovarian stimulation with rFSH associated or not with uFSH. Patients were split into two groups according with trigger: hCG ($n = 46$) or GnRH-agonist ($n = 81$). Oocytes were fertilized by ICSI, and all embryos were vitrified using standard protocols. For the frozen–thawed ET, endometrium preparation was performed with estradiol valerate plus vaginal micronized progesterone during 15 days. Embryos were thawed and evaluated for survival, morphology and transferred following standard protocols.
Main results and the role of chance: Laboratorial and clinical outcomes for hCG and GnRH-agonist groups were, respectively: patients age ($34.4 \pm 3.6 \times 33.3 \pm 4.4$; $p = 0.158$), FSH dose ($1722.6 \pm 312.1 \times 1611.2 \pm 321.8$; $p = 0.073$), MII oocytes collected ($13.0 \pm 4.9 \times 20.8 \pm 8.2$; $p < 0.001$), fertilization rate (79.4% × 81.5%; $p = 0.450$), number of embryos frozen ($9.7 \pm 3.8 \times 13.6 \pm 4.2$; $p < 0.001$) and post-thaw survival rate (91.8% × 95.6%; $p = 0.152$) and embryos transferred ($1.7 \pm 0.5 \times 1.6 \pm 0.5$; $p = 0.736$). In the frozen–thawed embryo transfer, patients who received hCG presented lower ongoing pregnancy rate (26.1%) than GnRH-agonist group (46.9%; $p = 0.021$). The logistic linear regression demonstrated that when GnRH-agonist was used, the chance of become pregnant is almost three times higher (OR: 2.8, $p = 0.033$), adjusted for patients age, dose of FSH administered, number of oocytes collected, fertilization rate, embryo survival rate and embryos transferred. No OHSS was observed.
Limitations, reasons for caution: Besides the retrospective characteristic of study, the number of patients is somewhat small and number of oocytes in the hCG group was smaller, showing much higher ovarian response in the GnRH-agonist group. On the other hand, we used multiple regression model to confirm the results adjusted for those possible confounders.
Wider implications of the findings: Hyper-responder patients who received GnRH-agonist trigger for final oocyte maturation plus all embryos-frozen can avoid OHSS, but the GnRH-agonist trigger also contribute to better oocyte quality, and hence a better prognosis in the following frozen–thawed ET. Our findings support the use of GnRH-agonist for final follicular maturation in hyper-responder patients.
Trial registration number: Not applicable.

P-683 Temporal effects of testosterone on expression of the circadian gene and steroidal acute regulatory protein gene in human luteinized granulosa cells

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Study question: How are the temporal effects of testosterone in expression of circadian genes and steroidogenesis related genes in cultured human luteinized granulosa cells?
Summary answer: Testosterone induces oscillating expression of the circadian gene PER2 and steroidal acute regulatory protein (STAR) gene in cultured luteinized human granulosa cells.
What is known already: Rhythmic expression of the circadian gene in granulosa cells can be induced *in vitro* in animal models. Moreover, circadian clocks may be involved in steroidogenesis in granulosa cells.
Study design, size, duration: Human luteinized granulosa cells were obtained from follicle fluid of ten patients during ovum aspiration undergoing IVF for each experiment which was replicated thrice.
Participants/materials, setting, methods: Accumulation patterns of circadian genes and steroidogenesis related genes mRNAs in cultured human luteinized granulosa cells were observed during 48 h after testosterone treatment by quantitative PCR.
Main results and the role of chance: The circadian genes CLOCK, PER2 and BMAL1 were expressed in cultured human luteinized granulosa cells. Among these genes, only expression of PER2 displayed oscillating patterns with a 20-h period in these cells after stimulation by testosterone. Expression of the other two genes did not show significant oscillating patterns. Expression of the STAR gene showed an oscillating pattern with a 16-h period. Testosterone repressed expression of CYP19A1 significantly but did not show apparently effect on expression of HSD3B2 and CYP11A1.
Limitations, reasons for caution: Mechanism under such phenomenon needs further study.
Wider implications of the findings: Circadian rhythm and steroidogenesis rhythm in human granulosa cells may be influenced by hormone signal from adjacent theca cells.
Trial registration number: not RCT

P-684 Comparative analysis of structural differences of GONAL-f and Bemfola: *in vivo* bioactivity and site-specific glycosylation mapping

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Study question: Are there any differences between biosimilar (Bemfola®) and originator (GONAL-f®) recombinant-human follicle-stimulating hormone (r-hFSH) based on *in vivo* bioactivity and on site-specific glycosylation mapping?
Summary answer: Bemfola's bioactivity was within the range stated in the product label, however, there are significant differences in glycosylation between Bemfola and GONAL-f or pituitary FSH.
What is known already: In the EMA assessment report, Finox declared the stated biological activity of 600 IU/ml from 526 to 648 IU/ml for Bemfola finished product, and from 555 to 595 IU/ml for GONAL-f, with a variance of 19% vs. 7% respectively. In addition, mapping of neutral sugars also indicates that Bemfola displays slightly more higher antennary structures than GONAL-f. On the other side it is well known that sugars at αAsn-52 affect bioactivity and that pituitary FSH does not show tetra-antennary sugars at this position.
Study design, size, duration: Bioassay: eight batches of Bemfola were assessed (PNS30388, PNS30230, PPS30400, PPS30021, PNS30390, PNS30228, PNS30389, PNS30229B; Expiry dates September 2016–September 2017), in order to evaluate biopotency range of Bemfola. Glycopeptide mapping: two batches of Bemfola were assessed (PNS30226, PPS30403; Expiry dates September 2016 and September 2017, respectively), and compared with three batches of GONAL-f (199F005, 197F049, 197F051; Expiry dates July–September 2017). Bemfola batches tested had 9–21 months to expiry.

Participants/materials, setting, methods: The *in vivo* bioassay for FSH potency (based on European Pharmacopoeia requirements) compared the effect of the test compound on ovary weight in immature rats vs. a reference standard r-hFSH preparation, which had been calibrated against the International Standard in International Units. Glycopeptide mapping was performed on the chymotryptic digest, and separation achieved with 0.1% TFA in acetonitrile/water on a HILIC column using Acquity UPLC coupled to Synapt G1 Mass Spectrometer (Waters, Milford, MA, USA).
Main results and the role of chance: The *in vivo* bioassay found that 6 out of 8 Bemfola batches showed higher bioactivity than expected, with activity up to 116.6%. In addition, significant differences were observed in the glycosylation patterns of Bemfola and GONAL-f. Tetra-antennary species, which have not been found in human pituitary FSH, were detected at αAsn-52 in in Bemfola (5.7–6.1%) whereas only traces (<1%) were observed in GONAL-f. A higher proportion of bi-antennary species at αAsn-52 was observed for GONAL-f (~75%) than for Bemfola (~53%). Furthermore, a lower degree of sialylation at αAsn-52 was observed for GONAL-f compared with Bemfola. A similar trend was observed at αAsn-78 and βAsn-7, with a greater proportion of bi-antennary species and a lower proportion of tri- and tetra-antennary species at these sites with GONAL-f compared with Bemfola. These results demonstrate that GONAL-f is structurally closer to pituitary FSH.
Limitations, reasons for caution: The analyses described were not performed in humans, and any clinical interpretations would need to be confirmed in a human model.
Wider implications of the findings: Glycosylation is a critical quality attribute of gonadotropins. The observed glycosylation differences between Bemfola and GONAL-f can impact both FSH signalling, gene expression profile and metabolic stability. The potential impact and clinical relevance of these differences on efficacy and risk of AEs should be further investigated in clinical settings.
Trial registration number: N/A.

P-685 Follicles environmental trace element pollution: Interaction and effects in infertile women undergoing ovarian hyperstimulation, and its correlation with IVF outcome

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Study question: What is the correlation and interaction of trace elements in women undergoing ovarian hyperstimulation and *in vitro* fertilization (IVF) and their correlation with pregnancy outcome?
Summary answer: Follicular microenvironment reflects environmental trace element exposure interactions having an impact on IVF outcome which can be increased by protective elements treatments prior to IVF.
What is known already: Environmental pollutants are found in highest concentrations in oocytes, sperm, ovaries and testes. These pollutants may lead to hormonal disorders and infertility by directly affecting the pituitary gland, causing abnormalities in sperm production and preventing ovulation and pregnancy. IVF outcomes are affected by toxic elements in follicular fluid levels. Reducing oocyte toxicity from lead, cadmium and nickel by concentrating the beneficial elements may counteract environmental pollutant toxicity. Pre-IVF mineral supplementation with protective elements may help to increase IVF success rates.
Study design, size, duration: A retrospective cohort study of 120 infertile women undergoing IVF were conducted. Participants were divided according to the cause of infertility into three groups: ovulatory, tubal, and unexplained infertility, and were compared to the male factor control group and the pregnant group. Trace element levels in serum and follicular fluid were measured to study the effect of toxic minerals and their interaction with each other in correlation with the IVF outcome.
Participants/materials, setting, methods: 120 women's serum and follicular fluid trace elements (Zinc, Copper, Selenium, Cadmium, Nickel, Cobalt, Lead)) were measured on ovum pick up day in women aged (22–44 years) who underwent IVF and ovarian hyperstimulation. Trace elements were measured using an Atomic absorption spectrophotometer. Statistical analysis was done using SPSS. Correlation matrix and Test were performed. The correlation between trace elements was analyzed. A comparison of their levels in the infertile, control, and the pregnant group was conducted.
Main results and the role of chance: There are trace elements interactions within certain mechanisms of depletion of toxic elements and concentrating the protective ones in the follicular fluid (FF) within follicles. Zinc levels ranged between 0.325 and 0.581 μg/ml in both serum and FF, Copper (1.011–1.363 μg/ml), Selenium (0.123–1.074), Cadmium (0.021–0.044 μg/ml), Nickel (0.53–0.074 μg/ml), Cobalt (0.049–0.061 μg/ml), Lead (0.046–0.07 μg/ml). Serum Zinc levels were half the normal value in all groups (0.054 ± 0.58 μg/ml), serum Copper levels were higher than FF in the infertile group (1.368 μg/ml). FF Selenium was highest in the control group (2.400 μg/ml). The highest levels of Cadmium were in the infertile group FF (0.44 μg/ml). Control group serum had the highest FF Nickel levels (0.082 μg/ml). The highest levels of Cobalt were in serum of the infertile group (0.06 μg/ml). The highest Lead levels were in the serum of the control group (0.096 μg/ml). The unexplained infertility group had the highest FF Cadmium & Lead levels 0.44, 0.53 μg/ml respectively, which was associated with high Progesterone levels in all infertile groups. Clinical pregnancy outcomes were 36% with 10% healthy deliveries. The highest pregnancy rate occurred in the control group and was associated with high FF Selenium level (2.38 ± 1.91 μg/ml), the lowest serum Cadmium levels (0.02 ± 0.01 μg/ml), higher FF Nickel levels (0.018 ± 0.24 μg/ml), the highest FF Cobalt levels (0.05 ± 0.03 μg/ml), and half the serum FF Lead levels (0.08 ± 0.01 μg/ml).
Limitations, reasons for caution: Infertile male partners were considered as the control group, since it is unethical for healthy women who don't want to have babies nor reserve their eggs to undergo ovarian hyperstimulation; or withdraw FF from healthy women who were unstimulated, thus healthy fertile women enrolled here served as a control.
Wider implications of the findings: IVF outcomes are affected by environmental pollution with toxic elements reflected in their FF and affected by ovarian stimulation by the presence of certain concentrating or depleting mechanisms within the follicle. Pre-IVF mineral supplementation with concentrated protective elements will counteract and reduce oocyte toxicity and increase IVF success rate.
Trial registration number: N/A.

P-686 Defining molecular mechanisms involved in the ovarian aging for clinical applications

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Study question: Our objective was to dissect molecular pathways of ovarian aging that result in menopause or premature ovarian failure to develop new strategies or treatment options.
Summary answer: Significant changes of gene expression were detected between young and aged ovaries, which could be target genes to treat or delay ovarian aging.
What is known already: Ovarian aging has been proposed one of the factors that are involved in pregnancy failure, and premature ovarian failure (POF) is also another cause of female infertility. Due to modern trends postponing child-bearing in women, ovarian aging increased pregnancy failure and other complications. Although several theories have been suggested such as mitochondrial malfunction, DNA damage/repair/methylation, caloric restriction, studies regarding ovarian aging-related molecular mechanism for development of therapeutic methods are insufficient so far.
Study design, size, duration: This is an experimental study that is consisted of two parts: in Phase I stage, we analyzed distinct gene expression profile between young and aged mouse ovaries, and in Phase II stage several preferentially expressed genes in both ovaries were selected and analyzed their physiological functions and involved molecular networks related to ovarian aging for development of diagnostic markers and therapeutic methods.
Participants/materials, setting, methods: Ovaries from 10-week- and 11-month-old FVB/NJ female mice with synchronized estrus cycle were collected for this study. A half of each ovary was used for RNA preparation and the other half for histological analysis. The expression profile analysis of young and aged ovaries was performed using the Illumina HiSeq 2000 System and functional annotation database-based gene-set enrichment analyses were employed

to evaluate aging-related molecular mechanisms. These findings were confirmed through qRT-PCR and immunohistochemistry.

Main results and the role of chance: We present the gene expression profile in young (10 weeks) and aged (11 months) ovaries by RNA-Seq. Based on Fragment per kilobase of transcript per Million mapped reads (FPKM) of each ovary from RNA-Seq experiments, 876 genes were identified that were preferentially expressed in young or aged ovary. Regarding molecular functions by GOTERM analysis, they were involved in growth factor binding ($p < 0.001$), cytoskeletal protein binding ($p < 0.001$) and platelet-derived growth factor binding ($p < 0.001$). In addition, these preferentially expressed genes code extracellular matrix (ECM) ($p < 0.001$), chromatin ($p < 0.001$) and nucleosome ($p < 0.001$)-related cellular components. In terms of biological process, nucleosome assembly ($p < 0.001$), regulation of hormone level ($p < 0.001$), chromatin organization ($p < 0.001$), response to reactive oxygen species ($p < 0.001$) and cellular macromolecular complex assembly ($p < 0.001$)-related genes might be involved in ovarian aging. Meanwhile according to KEGG Pathway analysis, signal pathways related to ECM-receptor interaction ($p < 0.001$), systemic lupus erythematosus ($p < 0.001$), focal adhesion ($p < 0.01$), steroid hormone biosynthesis ($p < 0.05$) and androgen/estrogen metabolism ($p < 0.05$) might play crucial roles in ovarian aging process. Further analyses are scheduled to produce transgenic animal models and with human ovarian tissues and cell lines.

Limitations, reasons for caution: This study does not include results from human materials, therefore it will be needed to verify their roles in ovarian aging using human ovarian tissues or cell lines for future clinical applications.

Wider implications of the findings: This study will provide new tools and enable formulation of new strategies to diagnose causes of ovarian aging and improve treatment options. It will be also providing new biomarkers useful to predict ovarian ages and reserve to care or rejuvenate aged ovaries.

Trial registration number: N/A.

P-687 GnRH-agonist triggering for final oocyte maturation in GnRH-antagonist IVF cycles induces decreased LH pulse rate and amplitude in early luteal phase: a possible luteolysis mechanism

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Study question: What is the mechanism of luteolysis in gonadotropin releasing hormone (GnRH) antagonist IVF cycles, triggered with GnRH agonist (GnRH-a) for final oocyte maturation?

Summary answer: Decreased baseline luteinizing hormone (LH) concentration, slowed pulse rate and reduced pulse amplitude may explain the mechanism of early luteal phase (ELP) luteolysis.

What is known already: GnRH-a ovulation triggering in GnRH-antagonist IVF cycles has been shown to reduce or even eliminate the incidence of ovarian hyperstimulation syndrome (OHSS) in high risk women. Following GnRH-a triggering menses can start as early as 4 days after ovulation, if no supplementation is given. The exact mechanism of this luteal phase insufficiency is still not fully understood.

Study design, size, duration: A prospective cohort study of ten patients who had IVF cycle with GnRH antagonist protocol, and were considered at high risk for developing OHSS (E_2 > 12,000 pmol/l and/or ≥15 follicles of ≥12 mm in diameter on trigger day). Ovulation was triggered with a bolus of 0.2 mg triptorelin when ≥3 follicles were ≥17 mm in diameter. Oocyte retrieval was performed 36 h later. Embryo transfer was performed 2 days post oocyte retrieval.

Participants/materials, setting, methods: Repeated blood samples were taken every 20 min for 6 h to measure serum LH, estradiol and progesterone. Women were divided into 2 groups: patients in group 1 ($n = 5$) were followed on the day of oocyte retrieval and patients in group 2 ($n = 5$) were followed 48 h after oocyte collection. LH pulse analysis was done with the deconvolution program. Pearson correlation coefficient was used to analyze hormone changes over time. Data are presented as mean ± SEM/SD

Main results and the role of chance: LH secretion: Mean LH and basal LH secretion rate were significantly higher in group 1 compared to group 2 (5.17 ± 1.97 IU/l vs. 0.616 ± 0.14 IU/l, $p = 0.049$) and (0.39 ± 0.036 IU/L/min vs. 0.0042 ± 0.0027 IU/L/min, $p = 0.0001$), respectively. The mean number of LH secretion pulses (0–2 pulses per women in 6 h follow up) was similar between the groups (1 ± 0.70 vs. 1.2 ± 0.83, $p = 0.48$) and the pulse amplitude was similar (1.13 ± 0.79 IU/l vs. 0.44 ± 0.44 IU/l, $p = 0.18$) for group 1 and 2 respectively. This LH secretion pattern represents a significant deviation from physiology where in ELP, pulse frequency rate is one pulse every 103 min and the mean LH pulse amplitude is 14.9 IU/l.

Estradiol (E_2): There was a significant decrease of E_2 over time in group 1 (5.442 ± 907 pmol/l to 3.376 ± 612 pmol/l, $r = –0.95$, $p < 0.00001$) and this decrease continued although more modestly in group 2 (3.036 ± 481 pmol/l vs. 2.642 ± 580 pmol/l, $r = –0.78$, $p < 0.01$).

Progesterone (P): There was a steady increase in P levels in group 1 (17.4 ± 3.73 nmol/l to 18.80 ± 5.80 nmol/l, $r = 0.53$, $p = 0.023$), followed by a sharp decline of P in group 2 (50.52 ± 8.97 nmol/l to 35.90 ± 6.25 nmol/l, $r = –0.94$, $p < 0.0001$). This decrease is in contrast to physiology where P levels increase to peak in the mid luteal phase.

Limitations, reasons for caution: The small number of participants (5) in each group.

Wider implications of the findings: Post GnRH-a triggering LH secretion pattern is aberrant in ELP, which may explain the dramatic luteolysis that follows. Luteal phase supplementation with intensive E_2 + P or LH (either LH itself or small hCG doses) is crucial to ensure receptive endometrium.

Trial registration number: NCT02449642.

P-688 Comparison of follicular fluid from natural and stimulated IVF cycles in the same women using antibody microarrays

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Study question: Is the protein profile in follicular fluid (FF) different in Natural Cycle IVF (NC-IVF) in comparison to conventional gonadotropin stimulated IVF (c-IVF)?

Summary answer: Sixteen out of 179 examined FF proteins were differently expressed in NC-IVF compared to c-IVF, suggesting gonadotropins to induce alterations in the FF protein profile.

What is known already: In c-IVF the implantation rate of unselected embryos is lower than in NC-IVF, which is possibly due to negative effects of the stimulation regimen on follicular metabolism. Accordingly, the endocrine follicular milieu and the concentration of putative markers of oocyte quality such as AMH have been shown to be significantly altered in FF by the gonadotropin stimulation in conventional IVF. These differences also suggest alterations in the FF protein profile induced by stimulation regimens in c-IVF. However, hardly any proteins with other functions such as cell signalling have been studied so far in this context.

Study design, size, duration: Retrospective cohort study involving 15 women who each underwent one NC- and one c-IVF treatment cycle between 2010 and 2015. c-IVF was performed by controlled ovarian stimulation with HMG and GnRH antagonists.

Participants/materials, setting, methods: Follicular fluid was collected from the leading follicles, and albumin and IgG removed with PROTIA immunoaffinity columns (Sigma). The flow-throughs (250 µL, >1 mg/mL) were labelled with Cy3 (NC-IVF) and Cy5 (c-IVF) reactive dyes (LKB), or *vice versa* to obtain a true duplicate. The samples were incubated on CSAA1 Panorama® arrays (Sigma) which contain antibodies against 224 proteins and which were then scanned for fluorescence at 552 (Cy3) and 650 (Cy5) nm. Normalisation required 6/21 array pairs.

Main results and the role of chance: Normalisation and standardisation was performed at several levels. First and foremost, the same women provided a NC- and a c-IVF cycle each. The Cy3/Cy5 dye swap allowed to remove staining bias. The arrays of 6 of the 21 couples were used to establish the normalisation algorithms. All protein signals were between those from negative and positive controls. Housekeeping proteins included 90 spots (45 proteins of the cytoskeleton), and the smoothing was applied over all spots with protein down weighting. The analysis of the selected 15 FF samples revealed 16 proteins which were found to show significantly ($P < 0.05$) higher ($n = 7$) or lower ($n = 9$) expression levels in NC-IVF when compared with c-IVF treatment cycles. These included cystatin A, DAP- and MAP-kinase and glutamic acid decarboxylase (NC-IVF higher than c-IVF), and active caspase-3, NF-κB and nerve growth factor receptor (NC-IVF lower than c-IVF).

Limitations, reasons for caution: Inclusion criteria were very strict to generate the most precise study results possible. Consequently the number of analysed samples was limited. The proteins with the highest differential expression will have to be quantified using single ELISA approaches.

Wider implications of the findings: The alteration of the protein profile could be a cause or a result of some metabolic differences induced by gonadotropins resulting in lower oocyte quality in c-IVF compared to NC-IVF. Cell signalling and apoptosis will be better understood with the identification of the most important players amongst the FF proteins.
Trial registration number: Not applicable.

P-689 Intramuscular progesterone (IM-P4) administration is associated with better pregnancy rates in frozen–thawed blastocyst transfers, regardless of progesterone serum levels

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Study question: Does IM-P4 administration 5–7 days before embryo transfer enrich the endometrium in a way that progesterone-in-oil enhances pregnancy rates in frozen–thawed embryo transfer cycles (FET)?
Summary answer: There is no difference in pregnancy rates according to P4 levels. However, IM-P4 route seems to induce better pregnancy outcomes.
What is known already: Many IVF practitioners' use high doses of IM-P4 in the luteal phase to enhance implantation in FET cycles. It appears that serum estradiol (E2) and P4 pave the endometrium in order to establish an artificially prepared lining. Luteal support is essential to maintain endometrium favorable to embryo implantation. Several P4 luteal phase support protocols for FET are routinely used and safely administered. There are P4 preparations available for vaginal, oral or intramuscular (IM) administrations with no clear correlation to serum levels or enhanced pregnancy rates.
Study design, size, duration: Retrospective study included 297 FET cycles between July 2014 and November 2015. Patients underwent endometrium preparation using E2 starting from 1st to 3rd day of menstrual cycle. P4 was administered when a proper endometrium was measured above 8 mm in thickness. P4 was placed vaginal ($n = 229$), vaginal + oral ($n = 30$) or vaginal + im ($n = 22$). Levels of progesterone were measured at the embryo transfer day. Patients were allocated into groups according to P4 levels as <10 ng/ml ($n = 59$), 10–20 ng/ml ($n = 157$) and >20 ng/ml ($n = 41$).
Participants/materials, setting, methods: All embryos were frozen at blastocyst stage. The blastocysts were warmed and checked for survival and quality; and one to four frozen–thawed embryos were transferred. The demographic characteristics of patients and pregnancy rates were compared among groups according to P4 route and P4 levels categories. Also, regression model was applied to assess the influence of P4 levels and route in pregnancy rates and adjusted for confounders.
Main results and the role of chance: Patients ages were 36.8 ± 4.4 with a mean of 1.9 ± 0.8 embryos warmed and 1.8 ± 0.6 embryos transferred. P4 levels according with P4 route groups were higher in the vaginal + IM group (vaginal: 16.9 ± 27.5; vaginal + oral: 14.0 ± 5.4; vaginal + im: 32.6 ± 44.2; $p = 0.039$), but there was no difference on endometrial thickness (vaginal: 9.0 ± 1.8; vaginal + oral: 8.9 ± 1.6; vaginal + im: 8.9 ± 1.2; $p = 0.996$). Patients who received P4 through vaginal + im route presented higher pregnancy rates (72.7%) than vaginal (51.5%, $p = 0.052$) or vaginal + oral (56.7%, $p = 0.013$). When patients were split into groups according to P4 levels, the clinical pregnancy rates were similar (P4: <10: 50.8%; P4:10–20: 56.1%; P4:>20: 53.7%; $p = 0.786$). A multiple logistic regression model were built to evaluate the P4 level or route in the pregnancy rates adjusted to patients age and number of embryos transferred. The P4 levels were not associated to pregnancy chance (OR: 1.0, $p = 0.852$), on the other hand, when patients received P4 vaginal + IM the chance of became pregnant was 2.5 higher and marginally significant (OR: 2.5; $p = 0.061$).
Limitations, reasons for caution: This is a retrospective study, without randomization according to P4 route of administration, and then the groups were not homogenously distributed. A multiple regression was applied to adjust for confounders, but a randomized prospective study would be necessary to better conclusions regarding differences in pregnancy according to P4 administration route.
Wider implications of the findings: Despite of subsequent higher progesterone levels, it seems that IM-P4 route, and do not progesterone levels itself, has a positive effect in pregnancy rates. It is known that IM-P4 can present a higher progesterone level stability and it might be the reason for those results.
Trial registration number: Not applicable.

P-690 The impact of excision of benign non-endometriotic ovarian cysts on ovarian reserve: a systematic review and meta-Analysis

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Study question: Does excision of benign non-endometriotic ovarian cysts cause damage to ovarian reserve as determined by Anti-Müllerian Hormone (AMH)?
Summary answer: Excision of benign non-endometriotic ovarian cysts seems to markedly reduce circulating AMH. It remains uncertain whether this reflects a real decline in ovarian reserve.
What is known already: Benign non-endometriotic ovarian cysts are very common and often require surgical excision. However, there has been a growing concern over the possible damaging effect of this surgery on ovarian reserve with potential compromise to fertility potential.

To date, several studies have investigated the impact of ovarian cystectomy on ovarian reserve as determined by circulating AMH. Most of these studies showed a postoperative decline in circulating AMH. However, given the relatively small size of these studies, further evidence is required to allow a firm conclusion.
Study design, size, duration: This meta-analysis included all cohort studies and randomized trials that analyzed changes of serum AMH concentrations after excision of benign non-endometriotic ovarian cysts. The included studies were conducted in the period between January 2000 and November 2015.
Participants/materials, setting, methods: The meta-analysis included nine studies with 305 patients who underwent excision of benign non-endometriotic ovarian cysts. Primary outcome was postoperative change in serum AMH level. Secondary outcomes were changes in postoperative serum follicle-stimulating hormone (FSH) concentration and antral follicle count (AFC).
Main results and the role of chance: Pooled analysis of 305 patients showed a statistically significant decline in serum AMH concentration after ovarian cystectomy (weighted mean difference (WMD) −1.00 ng/ml; 95% confidence interval (CI) −0.35 to −1.66), although heterogeneity between studies was high. Subgroup analysis including studies with a three-month follow-up, studies using Gen II AMH assay and studies using Immunotech (IOT) AMH assay improved heterogeneity and still showed significant postoperative decline of circulating AMH (WMD −1.50, −0.88, and −1.56, respectively). Sensitivity analysis including studies with low risk of bias and excluding studies with possible confounding factors still showed a significant decline in circulating AMH.
Limitations, reasons for caution: Given the high heterogeneity between included studies, this meta-analysis should be interpreted with caution. More well designed studies with long-term follow-up are needed, to draw a firm conclusion.
Wider implications of the findings: Women in reproductive age group presenting with benign non-endometriotic cysts should be counselled properly regarding the effect of surgical excision of ovarian cysts on ovarian reserve and reproductive performance in the future.
Trial registration number: None.

P-691 Granulosa cells of insulin resistance pcos women over express foxo 1 and it is linked with insulin signaling dysfunction

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Study question: ¿ Which is the status or the transcription factor forkhead box O1 (FOXO1) and its inactive isoform pFOXO1 in granulosa cells of Insulin Resistance PCOS women?
Summary answer: Granulosa Cells of PCOS–IR women over express FOXO1, the decrease of the inactivated form pFoxo1 in is related with the impairment of the Insulin signal.
What is known already: Metabolic disorders such as hyperinsulinemia, insulin resistance and obesity are features of Polycystic Ovary Syndrome (PCOS), with a relevant role in the pathogenesis of this disease. The transcription factor forkhead box O1 (FoxO1) plays roles in insulin-mediated glucose metabolism and have been linked to the pathogenesis of insulin resistance. Studies show that modulation of FoxO1 is essential for extra-hepatic insulin signaling to maintain glucose homeostasis. FOXO 1 is also expressed in granulosa cells (GC) and plays a critical role in promoting follicular atresia and cell apoptosis. However, it physiologic significance in reproductive tissue and PCOS women is unknown.
Study design, size, duration: A prospective case–control study conducted from March 2012 to June 2014. Local Institutional Review Board approved the study. Case: Insulin resistance PCOS (NHI) women: 20.

Control: Normal ovulatori women with male factor infertility as exclusive cause: 30.
Participants/materials, setting, methods: GC were issolated from the follicular fluid from NHI-PCOS-IR patients $n = 20$ and from normal women with male factor infertility control = 30 participants of the IVF program at our university center in Santiago. Insulin signaling in GC were evaluated measuring IRS1/p-IRS1, Akt/pAkt levels by Western blot. FoxO1/pFoxo1 levels and location were assesed by Inmunohistochemistry and Western blot GC were incubated with an Insulin-sensitizer drug Rosiglitazone Rz to asses the effect on insulin signaling pathway and FoxO1/pFoxo1 status.
Main results and the role of chance: PCOS GCs express higher levels of pIRS1 in serine residues (inactivating phosphorylation of insulin signaling) and low levels of total Akt and phospho Akt compared with control cells $p < 0.05$, reflecting and impairment in the insulin signaling pathway function. IR PCOS GCs express higher levels of FOxO1 and lower levels of pFoxO1. *In vitro* treatment with 0.1 µM Rz decreases IRS1serine phosphorylation and improve pAKT levels in PCOS CG. Rz decreases FOXO1 levels and improved pFOXO1 after stimulation with insulin in PCOS GC.
Limitations, reasons for caution: *In vitro* study, small sample.
Wider implications of the findings: These results may suggest that over expression FOxO1 in IR-PCOS-GCs is involved in the arrest of follicular development of PCOS, Insulinosensitizer administration as Rz can take important therapeutic rol, contributing to a better oocyte quality in this group of patients.
Trial registration number: None.

P-692 Impact of gonadotropin-type on progesterone elevation on the day of hCG during ovarian stimulation with corifollitropin alpha (CFA) or recombinant follicle-stimulating hormone (rFSH)

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Study question: Does hormonal stimulation in a GnRH-antagonist protocol, using CFA-only, has a lower incidence for premature progesterone rise on the day of hCG-trigger, compared to rFSH-only?
Summary answer: Controlled ovarian stimulation for IVF with CFA-only leads to a significantly lower incidence for progesterone rise on day of hCG-trigger, compared to stimulation with rFSH-only.
What is known already: An emerging body of evidence suggests that premature progesterone rise during the late follicular phase of stimulation for IVF has a negative impact on the outcome. However, the mechanism, by which the rise of progesterone reduces pregnancy rates is still not fully understood. It seems, that elevated progesterone does not have any negative impact on the oocyte or embryo quality. Its impact seems to be on the endometrium, leading to asynchrony between the endometrium and the developing embryo. Some studies suggest, that the cause of premature progesterone rise might be enhanced FSH stimulation in ART cycles.
Study design, size, duration: This study is a retrospective analysis of the data of the ENGAGE and PURSUE study, which were conducted from 2006 to 2008 and 2010 to 2012, respectively. Both were randomized, double-blind, double-dummy, active-controlled, non-inferiority trials, comparing CFA and rFSH during the first seven days of ovarian stimulation in a GnRH antagonist protocol. Data regarding stimulation medication used, stimulation duration, number of retrieved oocytes and incidence of elevated progesterone on the day of hCG-trigger were extracted.
Participants/materials, setting, methods: The ENGAGE and PURSUE trial included women aged 18–36 and 35–42 years, respectively, with a body weight of >60 – <90 kg and ≥ 50 kg, respectively, with a cycle length of 24–35 days and an indication for controlled ovarian stimulation before IVF. Patients were allocated to CFA-only or to rFSH-only-stimulation for the first 7 days. Blood was taken for hormonal assessment of progesterone on the day of hCG-trigger.
Main results and the role of chance: In the ENGAGE and the PURSUE trial, 1450 patients were randomized to CFA-treatment and 1446 patients to rFSH-treatment. Blood results of progesterone levels on the day of hCG administration were available from 1409 patients (CFA-treatment) and 1412 patients (rFSH-treatment), respectively. 5.4% (13/239) of patients that received CFA-stimulation and required no additional rFSH after day 8 to meet the criteria for final oocyte maturation, had progesterone level above 1.5 ng/ml compared to 18.5% (62/339) of patients that received rFSH-stimulation and no additional rFSH after day 8 ($P < 0.001$).

The analysis of patients with different ovarian response regarding the numbers of retrieved oocytes (0–5, 6–9, 10–13, 14–18, >18 oocytes) in regards to different stimulation-medications (CFA or rFSH) revealed the following incidence of premature progesterone rise: CFA-treated patients, without additional rFSH after day 8, had a 2.7–7.5% incidence of progesterone level above 1.5 ng/ml, independent from the number of retrieved oocytes (P = NS). In rFSH-treated patients without additional rFSH after day 8, the incidence of progesterone above 1.5 ng/ml levels was 6.3% (0–5 oocytes) and 31.6% (>18 oocytes) ($P < 0.001$).
Limitations, reasons for caution: The theory that step-down of FSH-stimulation-dosage in late follicular phase of controlled ovarian stimulation could lower the incidence of progesterone elevation has to be confirmed in future randomized controlled trials.
Wider implications of the findings: The pharmacokinetic profile of CFA is characterized by a rapid absorption, peak concentrations 2 days after injection, followed by decreasing FSH-activity, mimicking a step-down protocol. This reduces the thriving pressure on the growing follicle, resulting in a significantly lower incidence of premature progesterone rise, compared to daily stimulation with rFSH.
Trial registration number: The trials were registered under ClinicalTrials.gov identifier NTC00696800 (ENGAGE) and NCT01144416 (PURSUE).

P-693 does ulipristal acetate administration for symptomatic uterine fibroids impact ovarian reserve?

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Study question: The purpose of this study was to evaluate whether the use of ulipristal acetate to treat women with symptomatic myomas might compromises the ovarian reserve.
Summary answer: Using 3D-ultrasound technology and serum AMH concentrations we have been able to rule out a possible deleterious effect of ulipristal acetate on women ovarian reserve.
What is known already: Ulipristal acetate (UA) is a selective progesterone receptor modulator (SPRM) that presents a distinctive range of clinically advantageous properties in female reproductive tissues. Accordingly, the uterus is a significant therapeutic target for UA because of its action in reducing fibroid size and associated symptomatology. However, the possible impact of the UA on the ovarian reserve of premenopausal women has not been explored previously. Currently, this reserve can be evaluated by two different methods:

ovarian ultrasonography (Antral Follicle Count) and measurement of endocrine markers (Anti-Müllerian Hormone), as both have a notable agreement with histologically.

Study design, size, duration: A prospective longitudinal study was conducted at our Department of Obstetrics, Gynecology and Reproductive Medicine, from September 2013 to January 2016. The study was approved by the institutional review Board and all participants gave informed consent for the trial.

Participants/materials, setting, methods: The study was carried out involving premenopausal women ($n = 40$) with uterine leiomyomas who were judged to have a sufficient problem to justified surgical treatment. All the patients want to preserve fertility and therefore requested a conservative surgery (myomectomy). All patients were treated with UA (Esmya®) 5 mg daily before surgery for either 3 ($n = 20$) or 6 months ($n = 20$). 3D-ultrasound AFC, ovarian volume and AMH level were determined before and after the treatment with UA.

Main results and the role of chance: Serum AMH levels before (3.9 ± 0.2 ng/mL) UA were not statically significantly different from those after 3 months (4.1 ± 0.3 ng/mL) or 6 months (4.0 ± 0.2 ng/mL) treatment. In addition, no statistically significant difference in median Antral Follicle Count (28 ± 3.1 vs. 29 ± 2.8 vs. 27 ± 2.6) and median ovarian volume (7.06 ± 1.1 cm^3 vs. 6.89 ± 1.3 cm^3 vs. 7.09 ± 0.9 cm^3) were detected in the treatment groups between pretreatment 3D-ultrasonographic ovarian reserve evaluation and after 3 or 6 months of treatment with UA.

Limitations, reasons for caution: Our study do not conclusively rule out a possible detrimental effect on the ovarian reserve since this can only be achieved through an invasive and destructive method such as the remove of ovaries and carry out a histomorphometry-based follicle count.

Wider implications of the findings: Our findings can be useful when counselling young women with symptomatic myomas that desire to preserve fertility and we decided to treat them with ulipristal acetate before surgery. Consequently, ulipristal acetate is a safe treatment choice for these women as it does not compromise the ovarian reserve.

Trial registration number:.

P-694 Cluster analysis of metabolic risk factors in women of reproductive age: a prospective study

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Study question: Is there any association between endocrine disturbances and metabolic complications in women seeking gynecological care at their reproductive age?

Summary answer: Hyperhomocysteinemia (HHcy), followed by obesity, is the major determinant of cardio-metabolic disturbances in reproductive age women.

What is known already: The most well known correlation between metabolic syndrome (MS) and reproductive disorders is in women with polycystic ovary syndrome (PCOS). Recent reports suggest cardiovascular health in women is not improving as fast as that of men. Women of reproductive age often present with increased incidence of MS and cardiovascular disease (CVD) risk factors. Although studies of PCOS allied metabolic complications have been widely reported, the understanding of the correlation between endocrine status and metabolic complications in reproductive-age women remain limited and controversial.

Study design, size, duration: A prospective study including 1543 reproductive aged women was conducted for a period of 24 months. PCOS and MS were defined according to Rotterdam consensus and National Cholesterol Program Adult Treatment Panel III respectively. Participants were evaluated for MS and selected patients were randomly divided into two groups on the basis of high risk ($n = 143$) and low risk ($n = 711$) as classified by hierarchical cluster analysis by within-group linkage method.

Participants/materials, setting, methods: The participants were recruited at out-patient department of Institute of Reproductive Medicine, Kolkata. Serum concentrations of testosterone, insulin sensitivity and prolactin with metabolic parameters like C-reactive protein, lipid profile, homocysteine, sex-hormone binding globulin (SHBG), glucose and liver enzymes were evaluated between the two groups by chemiluminescence. Anthropometric measurements were recorded at the beginning of study. Odds ratio (OR) is computed. Poisson regression was used to generate risk ratio (RR) and 95% confidence intervals (CI).

Main results and the role of chance: For clinical diagnosis, 50.7% ($n = 433$) subjects had PCOS, 8.89% ($n = 76$) had hyperprolactinemia, 26.11% ($n = 223$) had tubal factor infertility and 3.39% ($n = 29$) had premature ovarian failure (POF). In terms of metabolic complications, 23.53% ($n = 201$) had MS, 11.12% ($n = 95$) had impaired glucose tolerance, and 4.8% ($n = 41$) had diabetes mellitus. Risk factors for metabolic disease are associated with a low age (<12 years) of menarche (RR, 1.26; 95% CI: 1.07–1.49) and a combination of high levels of C-reactive protein, homocysteine, and low levels of SHBG (RR, 1.44; 95% CI, 1.24–1.67). All metabolic indices excepting serum prolactin, alanine transferase and triglycerides were significantly ($p < 0.01$) different in high risk group. Hyperhomocysteinemia (>14 mmol/L) associated metabolic disturbance supersedes other clinical characteristics to set the strongest association between the two (OR, 20.1; 95% CI, 11.4–31.2). Overweight/obese status (OR, 11.2; 95% CI, 7.9–15.7) PCOS (OR, 1.6; 95% CI, 1.3–1.9), oligo/amenorrhea (OR, 1.3; 95% CI, 1.1–1.4), and hyperandrogenism (OR, 1.4; 95% CI, 1.2–1.6) were also found to increase the risk of cardiometabolic disease. However, tubal factor (OR, 0.7; 95% CI, 0.3–1), hyperprolactinemia (OR, 0.6; 95% CI, 0.3–1.1) and POF (OR, 0.3; 95% CI, 0.1–1.5) were not associated with the risk of cardiometabolic disease.

Limitations, reasons for caution: Although androgen excess may signal a risk for CVD, different types of androgens are not evaluated separately. Women were recruited from the outpatient clinic of a tertiary care center; hence, do not reflect true distribution of the general population. Therefore, results should be applied to the general population with caution.

Wider implications of the findings: Hyperhomocysteinemia was the major determinant of cardiovascular and metabolic disturbances in reproductive-age women. Even though many studies have shown an elevation in surrogate biomarkers of cardiovascular disease in PCOS women, it is still not clear as to what extent and magnitude the elevation precipitates more frequent and earlier events.

Trial registration number: Not applicable.

P-695 The different impact of stimulation duration on oocyte maturation and pregnancy outcome in fresh cycles with GnRH antagonist protocol in poor responders and normal responders

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Study question: What is the different impact of stimulation duration on fresh cycles in poor and normal responders during controlled ovarian stimulation (COS) using gonadotropin antagonist protocol?

Summary answer: Unlike normal responders, poor responders may have shorter stimulation duration with good oocyte maturation rate and clinical pregnancy rate.

What is known already: The gonadotropin stimulation is important for follicular growth, oocyte maturation, oocyte quality and endometrial development. Insufficient gonadotropin exposure may lead to nuclear or cytoplasmic immaturity of oocytes. On the other hand, prolonged stimulation may cause post-maturity of oocytes or apoptosis of granulosa cells and oocytes. It may also result in elevated progesterone and estradiol levels and impaired endometrial receptivity.

Study design, size, duration: This is a retrospective cohort study at a tertiary, university-affiliated medical center from January 2009 to June 2012. ICSI cycles using GnRH-antagonist protocol, without any pre-treatment, were included for analysis. Only the first stimulated cycles with fresh embryo transfer were included. The cycles with freezing all embryos were excluded from this study. A total of 443 women undergoing ICSI-ET cycle were included in this study.

Participants/materials, setting, methods: The starting dose of recombinant FSH was 200 IU with flexible antagonist protocol. The follicles reaching 18 mm with corresponding estradiol levels were used to determine hCG timing. Ovum pick-up number ≤3 were defined as poor responders ($n = 75$); ≥4 and ≤20 were normal responders ($n = 368$). End-points were oocyte maturation and clinical pregnancy rates. Both poor and normal responders were divided into four groups according to stimulation duration: ≤6, 7–8, 9–10, and ≥11 days.

Main results and the role of chance: The mean stimulation duration was significantly shorter in poor responders than normal responders (8.4 ± 2.0 vs.

9.0 ± 1.5, $p < 0.01$). Besides, the mean stimulation duration was significantly shorter in the pregnant group as compared with the non-pregnant group in poor responders (7.8 ± 2.4 vs. 8.5 ± 1.8, $p = 0.04$) and the mean stimulation duration was similar in normal responders (9.0 ± 1.5 vs. 9.1 ± 1.5, $p = 0.21$). Poor responders with a shorter stimulation duration seems to have a higher clinical pregnancy rate (≤6 days: 50.0%, 7–8 days: 31.3%, 9–10 days: 13.6%, and ≥11 days: 27.3%, $p = 0.19$), whereas the oocyte maturation rates were similar among them (≤6 days: 88.2%, 7–8 days: 85.0%, 9–10 days: 88.0%, and ≥11 days: 88.9%, $p = 0.98$). On the contrary, normal responders with the shortest stimulation durations (≤6 days) had the lowest clinical pregnancy rate (≤6 days: 27.3%, 7–8 days: 56.7%, 9–10 days: 47.5%, and ≥11 days: 50.0%, $p = 0.16$) and the lowest oocyte maturation rate (≤6 days: 69.0%, 7–8 days: 79.1%, 9–10 days: 82.1%, and ≥11 days: 78.3%, $p < 0.001$). Both poor and normal responders with shorter stimulation duration had a higher baseline estradiol levels and lower hCG-day progesterone levels ($p < 0.05$).

Limitations, reasons for caution: A larger sample size will be needed to demonstrate the proposed association between stimulation duration and ICSI outcome in poor and normal responders. With the retrospective nature of this study, it is inevitably influenced by some selection bias and the potential confounders related to COS and pregnancy outcome.

Wider implications of the findings: The higher baseline estradiol level may reflect earlier follicular recruitment by endogenous gonadotropin in late luteal phase. This may lead to relatively shorter follicular phase. The shorter stimulation in poor responders may reflect better granulosa cell function. Stimulation duration might be considered as adjunct parameters in determination of hCG timing.

Trial registration number: nil.

P-696 Putative functional link between FMR1 expression and mTOR/AKT–signal pathway during follicular control in human granulosa cells

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Study question: Assessment of *FMR1*/FMRP expression and the putative linked mTOR-AKT-signal-pathway in human granulosa cells modulated by FSH-stimulation.

Summary answer: FSH-stimulation activates *AKT-/mTOR*-expression but represses *S6K*- and *FMR1*-expression, while FMRP-expression increases during granulosa-cellular-growth. Results suggest a functional-link between them during follicular-maturation-process. Specific-pathway-inhibitior-assays support these findings.

What is known already: In rodents mTOR/AKT regulates early folliculogenesis by maintaining primordial follicles in dormancy induced by FSH for further differentiation and maturation. Consequently, FSH is used to stimulate oocyte-maturation in women for ART. Increased FSH-dosages are used in cases of diminishing ovarian reserve (DOR). Increased *FMR1*-expression in women carrying an *FMR1*-CGG_n-premutation ($n > 54$) in exon1 is known to reduce FMRP-expression and is associated with premature ovarian insufficiency/failure (POI/F). FMRP is mainly expressed in human granulosa cells within female ovary. As further studies suggest that CGG-triplet-numbers below or above $n = 26–34$ also affect ovarian reserve, the level of *FMR1*/FMRP-expression can be considered as novel ovarian-reserve-marker.

Study design, size, duration: Human COV434 granulosa-cell-line was used as model-system for FSH-stimulated cell-growth-control. Specific gene-expression-profiles of *AKT, mTOR and S6K,* as part of the mTOR-AKT-signaling pathway are compared to profiles of *FMR1*/FMRP before and after inhibiting specific mTOR/AKT-components.

Participants/materials, setting, methods: COV434 cell-line was treated with recFSH or the AKT-, mTOR-specific inhibitors MK-2206-2HCL and Rapamycin, respectively. TaqMan-analysis was performed to compare mRNA-expression-levels of *AKT, mTOR, S6K* and *FMR1* genes before and after FSH-stimulation and inhibitor-treatment. Protein-expression was determined using specific ELISAs. Statistical analysis including Mann–Whitney *U* and *T*-test were carried out with SPSS statistic software; statistical significance was set as $P \leq 0.05$.

Main results and the role of chance: Expression-profiles of *AKT, mTOR* in COV434 increased significantly ($P = 0.05$, $P = 0.015$ and $P = 0.05$) after treatment with recFSH. Surprisingly, *S6K*-expression decreased during the same treatment like *FMR1*-expression. After cell-line treatment with mTOR/AKT-inhibitors *FMR1*- and *S6K*-expression both significantly increased ($P = 0.031$). These findings suggest a putative functional link between expression of the mTOR/AKT signaling-pathway and the ovarian-reserve-marker-gene *FMR1* indicated by the *S6K*-expression level. Both are potentially involved in human folliculogenesis and oocyte maturation, as well as in ovarian reserve. Results are helpful to elucidate how FSH affects molecular mechanisms during follicular growth and under controlled ovarian stimulation. This suggests new starting points for functional research of human folliculogenesis and its disorders like POI/POF and DOR.

Limitations, reasons for caution: Presented description of a putative link of mTOR/AKT signal-pathway to *FMR1*-expression after FSH-stimulation is based on the use of the COV434-granulosa-cell-line-model. Before drawing conclusions for any clinical application these results have to be confirmed on primary granulosa-cell-cultures of women with different ovarian reserve.

Wider implications of the findings: Besides suggesting a potential functional link of *FMR1*- and mTOR-AKT-expression in human granulosa-cells, presented data offer novel starting points for further functional assays of human folliculogenesis. They reveal putative novel interferences between follicular maturation and molecular control to maintain the follicular pool after FSH stimulation.

Trial registration number: not applicable.

P-697 Fertility outcomes following single ideal blastocyst transfers in women less than 40 years of age as a function of ovarian reserve

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Study question:
Does ovarian reserve affect outcomes in single ideal blastocyst transfers in women less than 40 years of age?

Summary answer:
Whereas basal follicle stimulating hormone (FSH) levels had no impact, antral follicle count (AFC) and total gonadotropin dose used were correlated with live birth rates.

What is known already:
Several established methods are currently used to quantify ovarian reserve including: basal FSH levels, anti-mullerian hormone (AMH) levels, AFC, and total gonadotropin stimulation dose required. It is heavily debated whether decreased ovarian reserve represents a decrease in quality or quantity of oocytes, with the majority of data suggesting quantity is decreased and not quality. To test this theory, women with ideal quality single blastocyst transfers were evaluated when controlling for confounding effects, to see the role of ovarian reserve on live birth rates.

Study design, size, duration:
This is an ethics approved retrospective cohort study, which included 507 women undergoing their first ideal quality single embryo transfer between August 2010 and March 2014. Subjects were included only once. Analysis was done with logistic regression controlling for female age, duration of infertility, parity, Body Mass Index (BMI), and smoking status. It is the first study to evaluate the effect of ovarian reserve on pregnancy outcomes with ideal grade single blastocyst transfer.

Participants/materials, setting, methods:
The study was performed at a University Health Centre. Exclusion criteria included endocrine disorders, congenital uterine anomalies, endometrial polyps, intrauterine synechiae, adenomyosis, or hydrosalpinx. Subjects were stratified to normal or diminished ovarian reserve (DOR) on the basis of basal FSH levels (DOR ≥ 13 IU/L), basal AFC (DOR ≤ 8), and quartiles of dose of exogenous stimulation (DOR with increasing quartile). Basal analysis was performed between day 2 and day 5 of a spontaneous or induced menstrual cycle.

Main results and the role of chance:
In stratifying the subjects by AFC ≤ 8 ($N = 105$) to 8 ($N = 402$), the pregnancy rate (47% vs. 49%, $p = 0.25$) did not differ in these groups. However the

clinical pregnancy rate (32% vs. 40%, $p = 0.026$) and live birth rate (29% vs. 37%, $p = 0.037$) were superior in the group with a higher AFC. In repeating the analysis using basal serum FSH levels (<13 vs. ≥ 13 IU/L), the pregnancy rate (50% vs. 31%, $p = 0.27$), clinical pregnancy rate (40% vs. 13%, $p = 0.45$) and live birth rate (38% vs. 13%, $p = 0.48$) did not differ in these groups, when controlling for confounding effects. They do differ if confounders are not controlled for. A third analysis was performed using quartiles of FSH stimulation. The dose ranges were 200–1050 ($N = 127$), 1075–1575 ($N = 127$), 1600–2400 ($N = 127$) and 2425–7200 ($N = 126$) Units. The pregnancy rate ($p = 0.13$) did not differ in these groups. However, the clinical pregnancy rate ($p = 0.003$) and live birth rate ($p = 0.005$) were superior in the three groups requiring lower FSH doses combined, than the group which required the highest quartile of FSH to stimulate. The pregnancy rates in each quartile from lowest to highest were 45%, 52%, 54% and 41%. The clinical pregnancy rates were 36%, 43%, 47% and 25%.

Limitations, reasons for caution:
This retrospective study contradicts the assumption that ovarian reserve is solely a measure of quantity, not quality. The issue of quantity was bypassed with the initial selection of a population having produced ideal blastocysts, leaving quality as the remaining variable. Further evidence is needed, including relationship to AMH levels.

Wider implications of the findings:
The findings of this study suggest that ovarian reserve does affect quality of the embryo and outcomes when measured by AFC and exogenous stimulation but not by basal FSH levels. In this case, however, oocyte quality is measured by implantation potential and pregnancy outcome as opposed to embryo grade.

Trial registration number:
None.

P-698 The ultrasonographic "black crown": a new ovarian aspect pathognomonic of altered follicular ovarian status in infertile women

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Study question: To investigate whether the presence of an ultrasonographic hypoechogen crown surrounding the ovaries is associated with a specific profile of markers of the follicular ovarian status.

Summary answer: Present finding shows that the ovarian « black crown» is strongly associated with a reduced serum anti-Müllerian hormone (AMH) level and antral follicle count (AFC).

What is known already: Ovarian ageing is a natural phenomenon characterized by a continuous decay in the follicular stockpile and oocyte competence, thereby contributing to a gradual fertility decline. The reduction of the antral follicle cohort is best represented by decreasing levels of AMH. The identification of women who have severely decreased ovarian reserve for their age is clinically relevant, in particular for predicting response to ovarian stimulation in the assisted reproductive technology setting. Ultrasonographic antral follicular counting currently represents a pivotal marker of the follicular status. We recently identified an un-described aspect of the ovaries on ultrasound scan: the "black crown."

Study design, size, duration: Case–control study. From May 2015 to January 2016, 132 infertile normo-ovulatory women, 18 to 43 years of age, underwent transvaginal ultrasound scan and measurement of serum FSH and AMH levels. Ultrasound examination were performed by a two trained operators who were blinded to the results of hormone assays.

Participants/materials, setting, methods: We compared AFC and AMH values in 69 women showing the "black crown" sign and 63 age- and BMI-matched patients devoid of that ultrasonographic aspect.

The ovarian « black crown » was characterized by an hypoechogen, avascular, 1–2 mm thick aspect, completely or partly surrounding both ovariesPatients with a history of unilateral oophorectomy, ovarian surgery, or chemotherapy, as well as those displaying ovarian cysts were excluded of the present investigation.

Main results and the role of chance: As expected, by design, mean ages, duration of infertility and BMI were similar in both groups (35.3 ± 3.7 vs. 34.3 ± 3.9 years, $p = 0.13$), duration of infertility (32 ± 26 vs. 29 ± 29 months, $p = 0.13$), and BMI (22.5 ± 3.7 vs. 22.8 ± 3.8 Kg/m^2, $p = 0.6$). Interestingly, patients of the « black crown » group displayed shorter cycles when compared to controls. In addition, the presence of an ultrasonographic « black crown » was significantly associated with lower AFC (10.4 ± 5.2 vs. 21.3 ± 8.5, $p < 0.0001$) and reduced ovarian surface (3.5 ± 1.4 vs. 4.5 ± 1.9 cm^2, $p = 0.002$). On an hormonal standpoint, lower serum AMH levels were observed in women showing a "black crown" (1.2 ± 0.9 vs. 3.8 ± 3.1, $p < 0.0001$) while FSH values were higher (8.7 ± 4.2 vs. 6.8 ± 2.4, $p = 0.002$).

Limitations, reasons for caution: Our study was performed in a population of infertile normo-ovulatory patient. The sample size remain limited.

Wider implications of the findings:
Present findings shows that the new ultrasonographic sign "black crown" is tightly associated with reduced values of the ovarian reserve tests. Further prospective investigations are needed to determine intra- and inter-operator reliability. And whether the "black crown" is predictive of ovarian response to exogenous FSH and assisted reproductive techniques outcome.

Trial registration number: CEROG 2014-GYN-0302.

P-699 Levothyroxine replacement therapy for infertile patients with Hashimoto's disease may improve serum Anti-Müllerian hormone levels

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Study question: To investigate whether levothyroxine (LT4) supplementation for infertile patients with subclinical or overt hypothyroidism has an impact on Anti-Müllerian hormone (AMH).

Summary answer: After LT4 replacement, no significant change of serum AMH was recognized, but in the infertile patients with Hashimoto's disease, the AMH level was significantly increased.

What is known already: Thyroid dysfunction and thyroid autoimmunity are related to adverse impact on pregnancy as well as fertility. Subclinical hypothyroidism, aberrant high serum thyroid stimulating hormone (TSH) concentration with a normal free thyroxine level, is also responsible for subfertility. An elevated TSH level is associated with diminished ovarian reserve in women of reproductive age. However, the utility of LT4 replacement for subclinical hypothyroidism in infertile patients is still under discussion and a conclusion has not been reached.

Study design, size, duration: This study was approved by the Local Ethics Committee. Levels of serum thyroid-related hormones and AMH were measured in infertile women from 2014 to 2015 in Shimbashi Yume Clinic. Infertile patients with a high TSH level (>2.5 μIU/mL) were given a detailed thyroid examination in Ito hospital. We evaluated the relationship between AMH and TSH levels and the alteration of AMH levels in 1 and 3 months of LT4 supplementation during assisted reproductive technology treatment.

Participants/materials, setting, methods: Out of 1431 infertile patients, subclinical or overt hypothyroidism was found in 311 patients (21.7%). We excluded the patients with adverse factors on thyroid hormone and AMH, including polycystic ovary syndrome, premature ovarian dysfunction, treated thyroid dysfunction, ovarian tumor, post-ovarian surgery and smoking. Hashimoto's disease was detected in 37 patients out of the patients with elevated TSH level.

Main results and the role of chance: Serum AMH level correlated inversely with TSH concentration among all infertile patients. After LT4 supplementation, no significant change in AMH levels was detected in the patients with high TSH. The serum AMH level in the 37 patients with Hashimoto's disease (anti-thyroid peroxidase and/or thyroglobulin antibody-positive) was, however, significantly increased after treatment (1 month: 1.27 fold, $p < 0.001$ and 3 months: 1.20 fold, $p = 0.001$). Clinical pregnancy and miscarriage rates in infertile women with Hashimoto's disease were 65.2% (30/46 embryo transfer) and 20.0% (6/30 pregnancies).

Limitations, reasons for caution: The limitation of this study is that it is a retrospective study. Also, although we retrieved oocytes with spontaneous or minimum ovarian stimulation, infertility treatment may have an effect on AMH levels.

Wider implications of the findings: In basic research, thyroid hormone plays a significant role in folliculogenesis. Also, TSH and auto-immune thyroid antibodies have an adverse effect on follicle recruitment, leading to decreasing AMH. In the patients with Hashimoto's disease, LT4 treatment may support follicular development and relieve these adverse effects.
Trial registration number: None.

P-700 AMH levels variations during treatment with GnRh agonist: A prospective observational study

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Study question: Is Anti-Mullerian Hormone (AMH) a stable and reliable marker of ovarian reserve in women undergoing treatment with GnRH-agonist (GnRH-a)?
Summary answer: Dynamic changes in AMH levels occur up to 6 months after GnRH-a administration, suggesting that AMH is not a reliable marker in this population.
What is known already: AMH is a glycoprotein produced by granulosa cells of antral and preantral ovarian follicles. The AMH levels provide an indication of the size of the growing follicles pool and are commonly used as a surrogate biomarker of ovarian reserve. In young cancer patients, GnRH-a is administered during chemotherapy to preserve fertility, given that studies showed that it reduces the risk of ovarian failure by 57%. AMH levels are relatively stable along the menstrual cycle and during administration of the contraceptive pill, but conflicting data exist about its reliability in women undergoing GnRH-a treatment.
Study design, size, duration: From March 2013 to April 2015 women were enrolled in this observational study. According to a sample size calculation, based on previous studies on AMH levels in premenopausal women undergoing GnRH-a, with 65 participants, yet considering a standard dropout rate of 20%, a 95% study power with an alpha-error of 0.05 would have been achieved. Follow-up evaluation was concluded on November 2015.
Participants/materials, setting, methods: Sixty-nine patients waiting for surgery for uterine myoma, fibromatous uterus and endometriosis received 11.25 mg of GnRH-a. It was administered at 21th day of the menstrual cycle and repeated 3 months later at the University "Magna Graecia`-Catanzaro. At study entry and 1, 3 and 6 months after the administration of the first GnRH-a, serum levels of AMH, FSH, E2 and Antral Follicle Count were measured between first and fourth days of the menstrual cycle.
Main results and the role of chance: The mean (SD) age of participants was 31.9 (5.6) years. Mean body mass index (BMI) was 22.3 (3.5) kg/m^2. None had infertility. Average cycle length was 28 (2) days. At baseline the FSH and E2 mean levels were coherent with the women age. At the basal time the AMH mean (SD) level was 2.3 (0.6) ng/ml; one month later AMH level increased by 39% with a mean level of 3.8 (2.5) ng/ml ($P < 0.01$). At 3 months a significant decline of AMH levels [2 (0.8) ng/ml ($P < 0.01$)] was detected and this reduction became greater at 6 months [1.3 (0.7) ng/ml ($P < 0.01$)]. No significant differences in AFC were demonstrated 1 and 3 months after pituitary downregulation. Only at 6 months a significant AFC reduction was detected ($P < 0.01$). AMH showed a significant positive correlation ($P < 0.01$) with AFC at basal time, 1, 3 and 6 months after treatment. However, considering the significant increase of AMH and the stable number of antral follicles 1 month after pituitary downregulation, the ratio between AMH and AFC (evaluated at basal 1, 3, 6 month) was higher at 1 month after treatment than at the others evaluations. The suppression of FSH and E2 was maintained during the treatment period.
Limitations, reasons for caution: This study provides evidence that dynamic changes in AMH levels occur up to 6 months after GnRH-a administration, suggesting a partial AMH gonadotropin dependence. However, to understand the mechanism by which the GnRh-a acts on AMH clinical trials with longer follow-up and *in vitro* studies are needed.
Wider implications of the findings: In the context of cancer treatment, GnRH-a is often coadministered with chemotherapy with the aim of preserving fertility. To define the interference of GnRH-a on AMH levels is important for allowing the correct counselling in oncologic patients with reproductive desire.
Trial registration number: N/A.

P-701 No evidence for lower serum AMH levels in female BRCA1/2 mutation carriers

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Study question: Do *BRCA1/2* mutation carriers have a compromised ovarian reserve compared to proven non-carriers, based on serum anti-Müllerian hormone (AMH) levels?
Summary answer: *BRCA1/2* mutation carriers do not show a lower serum AMH level in comparison to proven non-carriers, after adjustment for potential confounders.
What is known already: It has been suggested that the *BRCA* genes are not only of importance in the prevention of cancer but also play a role in the process of ovarian reserve depletion. Previous studies have shown inconsistent results regarding the association between serum AMH levels and *BRCA* mutation status. Hence, it is yet unclear whether *BRCA1/2* mutation carriers may indeed be at risk of a reduced reproductive lifespan.
Study design, size, and duration: A multi-center, cross-sectional study was performed between January 2012 and February 2015 in 255 women. We needed to include 120 *BRCA1/2* mutation carriers and 120 proven non-carriers to demonstrate a difference in AMH levels of 0.40 μg/L (SD ± 0.12 μg/L, two-sided alpha-error 0.05, power 80%).
Participants/materials, setting, methods: Healthy women aged 18–45 years who were referred to the Clinical Genetics department and applied for predictive *BRCA1/2* testing because of a familial *BRCA1/2* mutation were asked to participate. A cross-sectional assessment was performed by measuring serum AMH levels and filling out a questionnaire. Multivariate linear regression analyses adjusted for age, current smoking, and current hormonal contraceptive use were performed on log-transformed serum AMH levels.
Main results and the role of chance: Out of 823 potentially eligible women, 421 (51.2%) were willing to participate, and of those, 166 (39%) did not meet our inclusion criteria. Two hundred fifty-five women were available for analyses; 124 *BRCA1/2* mutation carriers and 131 proven non-carriers. Carriers were significantly younger at study inclusion compared to non-carriers (median age: 29 [range 20–45] vs. 31 [range 18–44] years, respectively; $P = 0.02$). The median AMH level in carriers was 1.90 μg/L [range 0.11–19.00] compared to 1.80 μg/L [range 0.11–10.00] in non-carriers ($P = 0.34$). Adjusted linear regression analysis revealed no reduction in AMH level in the carriers (relative change = 0.98 (95% CI, 0.77–1.22); $P = 0.76$).
Limitations, reasons for caution: Participants were relatively young and power was insufficient to analyze *BRCA1* and *BRCA2* mutation carriers separately. AMH levels may have been influenced by the use of hormonal contraceptives, though similar proportions of carriers and non-carriers were current users and adjustments were made to correct for potential confounding in our analysis.
Wider implications of the findings: Currently, there seems to be no need to counsel healthy *BRCA1/2* mutation carriers on a jeopardized ovarian reserve condition. Larger, prospective follow-up studies with recurrent AMH measurements may be needed to really unravel a potential association between ovarian reserve and *BRCA* function.
Trial registration number: NTR no. 4324.

P-702 Neurokinin B receptor antagonism suppresses ovarian follicle growth and delays ovulation in healthy women

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Study question: Does pharmacological blockade of hypothalamic neurokinin B (NKB) signalling decrease gonadotropin (LH and FSH) secretion and suppress ovarian function in healthy women?
Summary answer: NKB blockade suppressed follicle growth and estradiol secretion, and delayed ovulation in women. NKB regulates female reproduction likely *via* the modulation of GnRH/LH pulse frequency.
What is known already: Normal follicle development and ovulation requires coordinated interaction between GnRH-driven gonadotropin stimulus to the ovary, and hormonal feedback. Neurokinin B (NKB) is a key modulator of GnRH secretion, as loss-of-function mutations result in hypogonadotropic hypogonadism. When administered throughout the menstrual cycle in women, a neurokinin B receptor antagonist delayed positive estrogen feedback and the LH surge, although no changes in basal LH secretion were seen. We have previously shown that NK3Ra decreased LH pulse frequency in normal women in a model of the mid-cycle LH surge.
Study design, size, and duration: We investigated the role of NKB in the control of follicle development in normal women using pharmacological blockade of NK3R. Six women were administered the NK3Ra, AZD4901, 40 mg orally twice a day for 7 days from cycle day 5–6. All women also had a no treatment control cycle, the order of cycles being randomised.
Participants/materials, setting, methods: Women were healthy with regular menstrual cycles. Serum hormones, leading follicle diameter and endometrial thickness were assessed through the follicular phase of treatment and control cycles until ovulation was confirmed by transvaginal ultrasonography. Urine was collected daily until next menses. Data were compared by ANOVA with Bonferroni multiple comparison post hoc analysis. Ethical approvals and informed consent were obtained.
Main results and the role of chance: Normal follicle development did not occur during NK3Ra treatment, the diameter of the leading follicle being significantly smaller than in controls at the end of treatment, i.e., on cycle day 12–13 (8.9 ± 0.8 vs. 14.5 ± 1.4 mm, $p < 0.02$). Serum estradiol was also lower (122 ± 57 vs. 406 ± 151 pmol/l, $p = 0.05$) and the endometrium was thinner (5.3 ± 0.4 vs. 7.6 ± 0.6 mm, $p < 0.04$) than in control cycles, although no clear differences in serum LH and FSH were observed. After treatment normal follicle development resumed (16.6 ± 1.4 vs. 16.9 ± 1.3 mm on day of LH surge, ns) and estradiol secretion increased (540 ± 105 vs. 608 ± 121 pmol/l, ns) with an LH surge on day 23 ± 2 vs. 15 ± 1 ($p < 0.02$). The delayed ovulation was confirmed by a similarly delayed day of peak urinary progesterone (cycle day 32 ± 2 vs. 22 ± 1, $p < 0.02$) and prolonged cycle length (37 ± 2 vs. 30 ± 2 days, $p < 0.04$); luteal function was unaffected by the NK3Ra (urinary progesterone 80 ± 17 vs. 61 ± 14, pmol/mol creatinine on surge day + 7, ns). To investigate further the mechanistic action of NK3Ra, a second group of women are currently undergoing frequent blood sampling analysis to investigate the effect of NK3Ra on LH pulsatility in the follicular phase.
Limitations, reasons for caution: Subtle changes in gonadotropin secretion were possibly not detected as intensive high-frequency sampling was not carried out. Such analysis is required to elucidate the hypothalamic action of NKB more precisely. One woman appeared to be a non-responder, and only one dose was investigated.
Wider implications of the findings: These findings demonstrate the involvement of NKB signalling in women in the physiological regulation of normal follicle development. Inhibition of NKB signalling has potential translational application in regulating GnRH/LH secretion in a wide range of applications, such as endometriosis, Polycystic Ovary Syndrome and in non-steroidal contraception.
Trial registration number: N/A.

P-703 Do not dismiss the long downregulation protocol for women at risk of ovarian hyperstimulation syndrome (OHSS)

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Study question: How effective and safe is the long downregulation protocol combined with low-dose gonadotrophin stimulation for women with above-average ovarian reserve?
Summary answer: In women with above-average ovarian reserve, the long downregulation protocol combined with low-dose gonadotrophin stimulation, results in satisfactory live birth and low OHSS rates.
What is known already: For expected "high responders," the use of a long downregulation protocol has been associated with similar live birth rates but higher incidence of OHSS compared to the antagonist protocol (Al-Inany et al., Cochrane Database Syst Rev 2011). As a consequence, current practice is moving away from prescribing the long downregulation protocol for these women.

However, the available randomized trials that compare the two protocols have utilised conventional stimulation doses (≥150 IU FSH daily). We hypothesized that using the long protocol with low starting gonadotrophin doses may constitute a safer but still effective stimulation regime for women at risk.
Study design, size, duration: This is a cohort study based on prospectively collected data from 473 consecutive women with above-average ovarian reserve (AMH ≥ 15 pmol/L), who were treated in a tertiary assisted conception unit from July 2013 to December 2014. During this period, we have been using an AMH-based algorithm for deciding the starting gonadotrophin dose. Women with above-average AMH levels were routinely prescribed the long downregulation protocol with a low starting gonadotrophin dose for their first IVF cycle.
Participants/materials, setting, methods: Women with normal-high (AMH 15–24 pmol/L) and high (AMH ≥25 pmol/L) ovarian reserve undergoing their first IVF/ICSI cycle, were prescribed the long downregulation protocol (from Day 21 of cycle) followed by a starting dose 100 IU–112.5 IU rFSH daily. A "freeze-all embryo" approach was recommended on the day of oocyte collection in the presence of 2 out of 3 risk factors (≥25 oocytes, ≥30 aspirated follicles, oestradiol ≥15,000 pmol/L on day of HCG).
Main results and the role of chance: The average age of women was 31 years old. In the group with normal-high AMH (144 women) a mean of 10 oocytes were retrieved, while in the high AMH group (329 women), a mean of 14 oocytes were retrieved. Suboptimal stimulation (≤3 oocytes) was experienced in only 4% of cases within each group.

The great majority of women (92%) had a single embryo (mainly blastocyst) transfer. The live birth rate was 47.2% (95% CI 38.7%–55.7%) per cycle and 50% (95% CI 41.2%–58.7%) per ET in the normal-high AMH group. In the high AMH group, the live birth rate was 38% (95% CI 32.6%–43.4%) per cycle and 43% (95% CI 37.1%–48.8%) per ET.

One case of severe late-onset OHSS and 3 cases of moderate late-onset OHSS occurred in the high AMH group (1.2% of cases), all eventually resolving and leading to live birth. In 7.9% of cases in the high AMH group, a "freeze-all embryo" approach was implemented. Of these, subsequent frozen embryo replacement achieved a 52% live birth rate.
Limitations, reasons for caution: As this is an observational uncontrolled study, it cannot provide insight to the comparative efficacy and safety of this modified regime against popular protocols for high responders, such as the antagonist protocol.
Wider implications of the findings: Previous research has associated the downregulation protocol with elevated OHSS risk in the context of conventional stimulation. This may not be the case if low gonadotrophin doses are utilised, as evidenced by the absence of significant early-onset OHSS and the infrequent cases of significant late-onset OHSS within our population.
Trial registration number: N/A.

P-704 The mechanism research on hyperhomocysteinemia-associated insulin resistance in polycystic ovary syndrome patients

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Study question: This study is to determine whether the level of homocysteine is associated with monocyte subtype related insulin resistance in women with polycystic ovary syndrome (PCOS).
Summary answer: Our results reveal that there is a positive relationship between homocysteine level and $CD14^{++}CD16^{-}$ inflammatory monocytes or insulin resistance in women with PCOS.
What is known already: The level of homocysteine is significantly higher in Chinese people than European or American people. Hyperhomocysteinemia

(HHcy) induced immune inflammation plays an important role in the pathogenesis of insulin resistance. Moreover, PCOS patients are often accompanied by HHcy, chronic low-grade inflammation and insulin resistance. Monocytes are important inflammatory cell subtype involved in insulin resistance.
Study design, size, duration: A cross-sectional study was carried out from December 2014 to June 2015 in Peking University Third Hospital, enrolling 108 PCOS-diagnosed Chinese women aged 21–35, among whom, 15 HHcy PCOS patients with homocysteine level exceeding 15 mmol/L, 93 PCOS patients with normal homocysteine level. We detected surface markers and related cytokines on the monocytes in peripheral blood samples, collected data of insulin resistance and levels of homocysteine.
Participants/materials, setting, and methods: Based on the Rotterdam PCOS criteria, 108 PCOS-diagnosed Chinese women aged 21–35 have been involved. We detected the monocyte surface markers and related cytokines in the peripheral blood samples by flow cytometry, collected data of insulin resistance and the levels of homocysteine. Confounding factors, including body mass index, blood lipid, and hormone were excluded from the analysis.
Main results and the role of chance: Compared with normal homocysteine group, HHcy patients have more $CD14^{++}CD16^{-}$ inflammatory monocytes and higher levels of IL-1β, IL-6, IL-12p70, and TNFα ($P < 0.05$) in the peripheral blood. Fast insulin and HOMA index, which are major factors reflecting insulin resistance degree, were increased in HHcy PCOS group. The results indicate that homocysteine level has a strong relationship with inflammatory monocytes, inflammatory cytokines, and insulin resistance.
Limitations, reasons for caution: Patient number in HHcy group was small, and sample size would be enlarged in the future study to confirm the conclusion.
Wider implications of the findings: Reducing the level of homocysteine could be one of the possible strategies in treating immune inflammation diseases associated insulin resistance commonly seen in PCOS patients, in order to improve the treating progress and prognosis of PCOS-accompanied insulin resistance.
Trial registration number: Not a clinical trial.

P-705 Low dose human chorionic gonadotropin as an adjuvant to GnRHa for final oocyte maturation rescues the poor outcome of suboptimal responders to GnRHa

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Study question: How about the pituitary responses to gonadotropin-releasing hormone agonist (GnRHa) trigger in gonadotropin and MPA (Medroxyprogesterone acetate)/progesterone (hMG + MPA/progesterone) treatment IVF/ICSI cycles, and can low dose of human chorionic gonadotropin (hCG) improve the outcome of patients with low pituitary response to GnRHa.
Summary answer: Lower basal LH is a risk factor for suboptimal response to GnRHa trigger. Low dose of hCG could rescue the poor outcome of suboptimal responder.
What is known already: Recently, we found that MPA was an effective oral alternative for the prevention of premature LH surges in woman undergoing controlled ovarian hyperstimulation (COH) for IVF. However, what is the optimal trigger strategy for the hMG+ MPA/progesterone treatment protocols still unknown.
Study design, size, duration: This is a retrospective analysis of a cohort of IVF/ICSI cycles ($n = 8970$) performed in university IVF center during the period from November, 2013 to December, 2015.
Participants/materials, setting, and methods: Eight thousand and ninety two women undergoing 8970 hMG + MPA/progesterone treatment protocol during IVF/ICSI cycles, and triggered with GnRH agonist alone or in combination with hCG (1000 IU, 2000 IU, or 5000 IU).
Main results and the role of chance: In total, 5.17% (464/8970) of patients exhibited a suboptimal response to GnRHa. The suboptimal responders had a significantly lower oocyte retrieval rate (55.83 ± 11.33% vs. 67.97 ± 23.66%, $P = 0.000$) compared with the appropriate responders. Suboptimal responders had significantly lower levels of basal FSH and LH, and FSH and LH levels on the day of trigger. Moreover, basal LH levels served as the single most valuable marker for differentiating the suboptimal responders with an AUC (the areas under the ROC curve) of 0.814. The dual trigger (GnRHa and hCG 1,000 IU), 34–36 h prior to oocyte retrieval, significantly increased oocyte retrieval rates (63.27 ± 24.45 vs. 56.42 ± 25.52, $P = 0.038 < 0.05$), mature oocytes rate (91.29 ± 15.92 vs. 85.09 ± 23.50, $P = 0.015 < 0.05$, and No. of embryos frozen (3.48 ± 2.70 vs.2.72 ± 2.13, $P = 0.039 < 0.05$) in patients with a suboptimal response. No case of OHSS occurred in the dual-trigger (GnRHa + hCG 1,000) group.
Limitations, reasons for caution: This is a retrospective study with its inherent limitations and bias. More sample size with balance in four suboptimal responder groups triggered with GnRH agonist alone or in combination with hCG (1000 IU, 2000 IU, or 5000 IU) is more valuable for analyzing.
Wider implications of the findings: The basal serum LH levels might also be useful predictor of the suboptimal response to the GnRHa trigger. Dual trigger using a low dose of hCG (1,000 IU) as an adjuvant to GnRHa for final oocyte maturation could improve the outcome of suboptimal responder.
Trial registration number: NA.

P-706 Intramuscular versus vaginal progesterone administration in medicated IVF frozen embryo transfer (FET) cycles: a randomised clinical trial

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Study question: To assess the effect of endometrial preparation with intramuscular progesterone compared to vaginal progesterone on subendometrial contractility in medicated FET cycles.
Summary answer: No difference was observed in subendometrial wave activity between intramuscular progesterone and vaginal progesterone in medicated FET cycles, despite a difference in serum progesterone concentrations.
What is known already: Intramuscular progesterone for luteal support has been popular in the United States, while in Canada and Europe vaginal progesterone is routinely used. Increased frequency of subendometrial contractions in fresh IVF cycles has been associated with lower pregnancy rates and exposure to exogenous progesterone may reduce the frequency of subendometrial contractions. Meta-analyses showed that pregnancy outcomes were similar between intramuscular and vaginal progesterone luteal support in fresh IVF cycles. Less data exists about intramuscular and vaginal progesterone effects on subendometrial contractions and pregnancy rates in frozen embryo transfer cycles. Subendometrial contraction frequency was the primary outcome measure of the present study.
Study design, size, and duration: This was a randomised clinical trial including 34 patients undergoing thawed embryo transfer at the blastocyst stage. Patient allocation to vaginal or intramuscular progesterone was performed by a third party using a computerized randomization table. Sample size calculation based on a difference of 1.8 endometrial waves per minute (variance in Fanchin's original study), assessed by transvaginal ultrasound on the day before the embryo transfer, determined 15 subjects in each arm was required for $P < 0.05$.
Participants/materials, setting, and methods: Patients were treated by the standard medicated FET protocol in our centre, which included oral micronized 17b-estradiol starting on day 3 of the cycle. Once sufficient endometrial proliferation (>7 mm with a triple-line pattern) was achieved, the patient started progesterone. Patients in the vaginal progesterone arm were treated with 200 mg vaginal suppositories 3 times daily. Patients randomized into the intramuscular (IM) progesterone arm were treated with once daily injections of 50 mg progesterone in oil.
Main results and the role of chance: The 17 patients in each study arm were similar in age (35.6 ± 3.7 years in the IM group, 37.1 ± 7.1 years in the vaginal group) and in Day 3 serum estradiol levels (149.1 ± 86.1 pmol/L and 137.0 ± 142.1 pmol/L, respectively, $P = 0.8$). The IM progesterone group had lower day 3 FSH (5.2 IU/l ± 1.5 IU/L) compared to the vaginal group (9.6 IU/l ± 12.1 IU/L, $P < 0.05$). As expected, serum progesterone levels were higher in the IM group compared to the vaginal group on the day of the wave study (85.2 ± 50.1 nmol/L vs. 30.3 ± 11.2 nmol/L, respectively, $P = 0.005$). The number of subendometrial waves counted by ultrasound (blinded examiner) in the IM progesterone group (2.4 ± 4.8 waves/min) was not significantly different from the vaginal progesterone group (1.4 ± 1.1 waves/min; $P = 0.4$). The pregnancy rate was 53% for both groups. In a logistic regression model, both higher FSH levels and higher wave frequency (combining both treatment groups) were significantly correlated with a compromised outcome.

Limitations, reasons for caution: Although this study was adequately powered to test the differences in subendometrial contractility frequency, according to wave frequency parameters related to pregnancy rates chosen from previous work, the number of subjects was still small. A larger study may have shown a difference in contraction frequency between IM and vaginal progesterone.
Wider implications of the findings: The study group consisted of relatively older patients going through a blastocyst transfer and therefore the results should be generalized with caution to cellular stage transfers and younger patients. Higher subendometrial wave frequency appears to be associated with poorer clinical pregnancy outcome as previously shown by other studies.
Trial registration number: ClinicalTrials.gov NCT02078869

P-707 Assessment of endometrial thickness in women with luteinizing hormone deficiency undergoing ovulation induction

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Study question: Does peak endometrial thickness (ET) impact on pregnancy rates in women with WHO type 1 anovulation undergoing ovulation induction (OI)?
Summary answer: Pregnancy rates are significantly higher in those women with a peak ET greater than 6 mm.
What is known already: Optimal endometrial development is essential for embryo implantation. Folliculogenesis with adequate oestradiol biosynthesis drives endometrial proliferation. It has been widely demonstrated that in women undergoing IVF treatment, ET at the time of embryo transfer can affect clinical pregnancy rates. Less attention has focused on women diagnosed with type 1 anovulation with gonadotrophin deficiency. A challenge in this population, undergoing ovulation induction with exogenous gonadotrophins, is suboptimal endometrial development despite clinical folliculogenesis. This scenario is a feature of inadequate stimulation by Luteinizing Hormone (LH).
Study design, size, duration: This is a single centre retrospective cohort study. Women with a diagnosis of WHO type 1 anovulation attending the Reproductive Medicine Unit at the University College London Hospital between 2006 and 2015 and embarking on OI with human menopausal gonadotrophins (hMG) were included in the study.
Participants/materials, setting, methods: 87 women were included in the study. Demographic, clinical and ultrasonic data at baseline and during OI were obtained by reviewing medical records. A total of 261 treatment cycles were identified of which peak ET was recorded in 225.
Main results and the role of chance: 87 women with type 1 anovulation underwent ovulation induction with hMG. 37 women were diagnosed with Hypothalamic Amenorrhoea (HA), 35 Hypogonadotrophic Hypogonadism (HH) and 15 with Hypopituitarism (HP). There was no difference in baseline characteristic with reference to age ($P = 0.94$) or baseline ET ($P = 0.23$). Women diagnosed with HA had a significantly lower BMI compared with the other diagnostic groups ($P = <0.01$).

Of the 225 cycles, 78 were completed for women with a diagnosis of HA (34.7%), 95 HH (42.2%) and 52 HP (23.1%).

An ET ≥ 6 mm was achieved in 194 cycles (86.2%), whilst an ET <6 mm was demonstrated in 31 cycles (13.8%) and this was equally distributed across diagnostic subgroups. Peak ET was not affected by baseline characteristics.

58/225 (25.8%) cycles resulted in clinical pregnancies, 21/58 (36.2%) were women with a diagnosis of HA, 23/58 (39.7%) HH and 14/58 (24.1%) HP.

56/194 (28.9%) of women who demonstrated a peak ET ≥6 mm achieved clinical pregnancy, compared to 2/31 (6.5%) where the ET was <6 mm ($P = 0.004$). Baseline variables or cycle number did not affect the outcome.
Limitations, reasons for caution: This is a retrospective cohort study and is reliant on the quality and quantity of the data entry at the time of clinical treatment. Furthermore, inter-observer bias needs to be considered, as a single observer did not conduct all the ultrasound examinations.
Wider implications of the findings: Research to date has focused mainly on IVF populations who exhibit supraphysiological oestradiol concentrations during stimulation. In contrast, different mechanisms underpin thin endometrium in women with type 1 anovulation and these women may be at particular risk of thin endometrium depending on the LH content of gonadotrophins used for stimulation.
Trial registration number: The Institutional Review Board of the UCLH was contacted to obtain approval for this study and formal ethics approval was not required.

P-708 Identification of altered microRNAs and mRNAs in cumulus cells of PCOS patients: miRNA-509-3p promote estradiol secretion by targeting MAP3K8

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Study question: Our aim was to identify the altered miRNA and mRNA expression profiles in cumulus cells of PCOS patients to research their molecular function in the abnormal folliculogenesis of PCOS.
Summary answer: miR-509-3p was up-regulated and MAP3K8 was down-regulated expressed in PCOS cumulus cells. miR-509-3p could improve E_2 secretion by inhibiting the MAP3K8 expression directly.
What is known already: Although the clinical and biochemical signs of PCOS are typically heterogeneous, anovulation which often induced by abnormal folliculogenesis is considered to be a common characteristic of PCOS. The possible mechanisms of anovulation in PCOS have been proposed as follow: high levels of estradiol in PCOS patients could prevent an increase in follicle-stimulating hormone (FSH), which is an essential factor for follicular growth and ovulation induction. The low level of FSH will consequently lead to anovulation. Until now, many genes were identified to elucidate the pathophysiology of abnormal folliculogenesis in PCOS, but how these genes are post-transcriptionally regulated is poorly understood.
Study design, size, duration: miRNA and mRNA expression profiles of the cumulus cells isolated from PCOS and control patients were determined by miRNA and mRNA microarrays. The microarray data were validated by qRT-PCR. The potential target genes of altered miRNAs were predicated by miRWalk 2.0. By compared the list of potential target genes to the list of DEGs in our mRNA microarray, the candidate miRNA and its target genes were chosen for further research in KGN cells.
Participants/materials, setting, methods: A total of 36 participants (18 PCOS and 18 controls) were included in this study. miRNA and mRNA expression profiles of the cumulus cells isolated from 5 PCOS and 5 control patients were determined by miRNA and mRNA microarrays. The cumulus cells isolated from others patients were used in qRT-PCR and western blot analysis. The regulation of candidate miRNA on target genes was studied by luciferase activity assay and transfection tests.
Main results and the role of chance: From the microarrays data, 17 miRNAs and 1263 mRNAs showed significantly different expression in PCOS cumulus cells. The microarrays data were confirmed by qRT-PCR. Compared the list of DEGs isolated from cDNA microarray and the list of predicted miRNA targeted genes, we found that several miRNA targeted genes (i.e., MAP3K8, miR-509-3p target gene; RND3, miR-200b-3p target gene) were also identified by the mRNA microarray. Specially, miR-509-3p was also identified by recently published reports of altered miRNAs in PCOS cumulus cells, with almost the same fold changes as our results. So, miRNA-509-3p and its potential target gene (MAP3K8) were selected for further researched. It showed that miR-509-3p was up-regulated and MAP3K8 was down-regulated expressed in PCOS cumulus cells. The directly interaction between miR-509-3p and MAP3K8 was confirmed by luciferase activity assay in KGN cells. In addition, the miR-509-3p mimics or inhibitor transfection tests in KGN cells further confirmed that miR-509-3p could improve E_2 secretion by inhibiting the MAP3K8 expression. These results will offer new insight into the pathogenesis of anovulation in PCOS, especially the regulation of estradiol production.
Limitations, reasons for caution: We used microarray to identify the altered miRNA in the present study. By using this kind of method, only the miRNAs with its response to probes which included in the microarray could be detected and novel miRNAs could not be found.
Wider implications of the findings: We found that miR-509-3p and its target gene (MAP3K8) showed differentially expressed in PCOS cumulus cells. Further researches in KGN cells confirmed that miR-509-3p could improve E_2 secretion by inhibiting the MAP3K8 expression. These results will offer new insight into the anovulation in PCOS, especially the regulation of estradiol secretion.

Trial registration number: The study belongs to basic research and has no registration number.

P-709 Mechanism of androgen excess inducing neuroendocrine disorders in female rats and dysfunction of GnRH secretion in GT 1-7 cell

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Study question: Neuroendocrine Disorders of Rats with Ovulation dysfunction Induced by Androgen Excess.
Summary answer: Our study has showed that there are neuroendocrine disorders in PCOS models and hyperandrogenism could inhibit the neuropeptide secretion of neuron cell line.
What is known already: Polycystic ovary syndrome (PCOS), which is characterized by ovulatory dysfunction, hyperandrogenism, insulin resistance and neuroendocrine dysfunction, is the most common reproductive endocrine disorder in young women. Although the pathogenesis of neuroendocrine disorder in PCOS is still complex and controversial, a number of relevant studies have emerged to clarify the possible causes. The clinical studies have estimated that inappropriate GnRH secretion with accelerated LH and low FSH level is the typical symptom in PCOS development.
Study design, size, duration: The female rats of 3 weeks aged were used in this study as PCOS animal model. We observed the model from 3 weeks age to 12–15 weeks age and the neuron cell line-GT1-7 cell was including in this study as cell model. Western blot, quantitative PCR, fluorescence staining and Elisa were used.
Participants/materials, setting, methods: The female rats of 3 weeks aged were implanted subcutaneously the silicone tube containing 7.5 mg DHT or nothing as control. Then the serum steroid hormones and estrus cycle were observed pubescent aged and adult aged female rats, and the expression level of endocrine factors in hypothalamus were tested in both groups. Moreover, we observed the effect of DHT and Endoplasmic reticulum stress inducer-TG on GnRH secretion in mice hypothalamus neuroma cell line-GT1-7 cell.
Main results and the role of chance: Comparing to controls, the body weight of DHT rats was significantly higher ($p < 0.01$); and the number of cystic and atretic follicles in DHT rats' ovaries was markedly more than that of controls. Moreover, there was no regular estrus cycle and LH frequency observed in DHT rats that were 8-week-old. Our data showed that the DHT rats of 4–8 weeks of age presented the significant higher expression level of ER stress markers and lower expression level of Kisspeptin in hypothalamus than that of controls ($p < 0.05$), which mean that DHT up-regulated the expression of ER stress signal, and inhibited the Kisspeptin expression. Moreover, in GT1-7 cell line, both of DHT and TG could significantly block the GnRH secretion, which Kisspeptin could up-regulate ($p < 0.05$). All the results suggested that the secretion of GnRH in neuron cell could be inhibited by DHT and ER stress, meanwhile, Kisspeptin could block the effect of DHT and TG.
Limitations, reasons for caution: Only one kind of animal and cell model was used, and the simple of PCOS patients were not including in this study.
Wider implications of the findings: Our data showed that the androgen excess can induce dysfunction in rat models, decrease of Kisspeptin secretion in cell line.
Trial registration number: 2012-SR-048

P-710 The effect of elevated late follicular phase serum progesterone on IVF outcome in good prognosis patients

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Study question: Does elevated progesterone on the day of HCG affect IVF outcome after accounting for important confounding variables in women with expected good ovarian response?
Summary answer: Elevated serum progesterone level is associated with lower clinical pregnancy rate. However, this result was not reproduced when top quality blastocyst was transferred.
What is known already: Previous studies have reported conflicting results with regard to the effect of high progesterone level in the late follicular phase on IVF outcome. However, there is a wide variation in the population studied, with little evidence available to confirm a detrimental effect of elevated serum progesterone level in the late follicular phase on the clinical pregnancy rate in cycles where a normal or high ovarian response is expected, and particularly after the transfer of high quality blastocysts.
Study design, size, duration: This is a prospective cohort study carried out between May 2014 and December 2015 at a tertiary Assisted Conception Unit in a teaching Hospital. A total of 615 fresh IVF/ICSI cycles were included in the study.
Participants/materials, setting, methods: Women with predicted normal and high ovarian response based on the total AFC (≥10) at baseline scan were recruited.

Exclusion criteria include 1) PGD and oocyte donation, total embryo freezing and when GnRH agonist was used to trigger ovulation. Serum progesterone concentration was measured on the day of HCG administration. Patients were grouped according to their serum progesterone concentration into (high $P \geq 5$ pmol/L) and (normal $P < 5$ pmol/L).
Main results and the role of chance: A total of 615 patients were analysed. 125 (20.3%) had elevated progesterone. There were no statistically significant differences between the 2 groups with regards to age (34.8 ± 4.3 vs. 34.1 ± 4.0, $p = 0.1$); baseline AMH (30.4 ± 26.2 vs. 33.7 ± 25.1, $p = 0.2$); number of embryos transferred (1.56 ± 0.63 vs. 1.51 ± 0.58, $p = 0.5$) and the proportion of top quality blastocysts transferred (65% vs. 61%, $p = 0.3$). Cycles with high Progesterone had significantly higher AFC (17.3 ± 6.9 vs. 18.9 ± 7.5, $p = 0.02$), total FSH dose (2710 ± 1082 vs. 2172 ± 1170, $p < 0.0001$), longer duration of stimulation (13 ± 1.8 vs. 12 ± 2.9, $p = 0.002$), oocyte (15.1 ± 6.3 vs. 13.4 ± 6.1, $p = 0.006$) and 2PN number (9.2 ± 4.5 vs. 7.7 ± 4.2, $p = 0.0007$), A multivariate analysis accounting for important confounding variables (e.g., AFC, oocyte number) showed a significantly lower clinical pregnancy rate CPR (30.4% vs. 43.7%) in the group with elevated progesterone (OR: 0.59, 95% CI: 0.38–0.93, $p = 0.021$). However, a subgroup analysis where at least one top quality blastocyst was transferred showed no difference between the two groups (44% vs. 50%, $p = 0.24$).
Limitations, reasons for caution: Although this is a large prospective study with strict inclusion criteria, it is a single-centre study using baseline AFC to select women with predicted normal or high ovarian response during IVF/ICSI treatment. The results may not apply to other groups of women with different ovarian reserve parameters.
Wider implications of the findings: It is yet to be determined which group of patients with high progesterone is unlikely to achieve a pregnancy. Patients with a top quality blastocyst transfer and high progesterone may achieve comparable results to their peers with normal progesterone concentration.
Trial registration number: None.

P-711 High level of androgen during controlled ovarian stimulation cycle impairs endometrium receptivity in PCOS patients

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Study question: Does High level of androgen during COS cycle impact fresh embryos implantation in PCOS patients?
Summary answer: High level of androgen during COS cycle impaired endometrium receptivity, which contributed to the low fresh embryo implantation rate in PCOS.
What is known already: Hyperandrogenism is the main pathologic characteristic of polycystic ovary syndrome (PCOS). It is generally believed that poor reproductive success in IVF could be achieved in PCOS patients compared with infertile women with tubal factor.
Study design, size, duration: 1357 infertile patients undergoing first IVF cycle treatment were recruited from January, 2014 to June, 2015 in this case control

study. Among these subjects, 177 women were PCOS and 1315 were Controls matched for BMI and age.
Participants/materials, setting, methods: Patients with PCOS were treated with OC before IVF cycle. Standard controlled ovarian stimulation was performed in both of groups. Serum hormone was detected on day 3 and HCG day during the COS cycle. The expression levels of IGFBP-1, EGF, LIF, etc., were screened in endometrium from both groups using real-time PCR. The mRNA and protein expression of IGFBP-1 and LIF were examined in Ishikawa cells treated with different concentrations of testosterone.
Main results and the role of chance: In the total study population, serum LH, E2 and total T levels on day 3 in women with PCOS were comparable with Controls, and no significant differences in the number of oocytes retrieved, fertilization rate, available embryos rate and high quality embryo rate between two groups. While the implantation rate, clinical pregnancy rate, on-going pregnancy rate and live birth rate in PCOS group was obviously decreased compared to the controls. In the subgroup of 154 patients, total T level on hCG day was significantly higher in PCOS (n = 27) than in Controls (n = 127). By endometrium biopsy we found that the mRNA and protein levels of IGFBP-1 and LIF of women with PCOS were significantly lower than in those of controls. In Ishikawa cells, we found high dose of testosterone (more than 10–8 mol/L) significantly reduced the IGFBP-1 and LIF mRNA and protein levels.
Limitations, reasons for caution: The mechanism of how elevated androgen level causes expression of LIF and IGFBP1 reduced hasn't been proved in this study. Further study was needed to clarify it in later study.
Wider implications of the findings: Although endocrine abnormal including hyperandrogenism had been corrected before COS cycle started, higher androgen level was detected on HCG day of COS cycle in PCOS patients, which probably contributed to the decreased fresh embryos implantation rate.
Trial registration number: ChicCTR-OCH-14004536.

P-712 Extensive analysis defines actual differences in absolute and "per-follicle" Anti-Müllerian hormone (AMH) levels obtained by manual and new automated assays

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Study question: To compare absolute and "per-follicle" AMH levels, as well as the strength of their relationship with antral follicle count (AFC), obtained with 2 automated and 1 manual commercially-available assays.
Summary answer: Although strongly correlated with AFC, AMH levels were lower with automated (−16% and −20%) than with manual assays but higher (5%) with Access than Elecsys.
What is known already: Associated or not to AFC, serum AMH measurements have become the paramount reference in the appraisal of ovarian follicular status. Yet, the observation of non-reproducible and/or aberrant AMH results, often resulting from different calibration, standards, and operator manipulations, discouraged its widespread use. Recently, fully automated AMH assays have become available but extended analysis testing their reliability – in particular taking the relationship between AMH and the number of AMH-producing follicles as reference – remains necessary.
Study design, size, duration: Frozen-thawed serum aliquots obtained between days 2 and 4 of the menstrual cycle in 211 assisted conception candidates aged 24 to 43 years between April 2015 and July 2015 were analyzed. All of them had concomitant, centralized assessment of AFC (follicles measuring 3–10 mm in diameter). "Per-follicle" AMH levels were pragmatically appraised by dividing serum AMH levels by AFC (results expressed in ng/mL/follicle).
Participants/materials, setting, methods: Serum AMH levels were determined using 3 different assays: modified Gen II AMH ELISA (Beckman Coulter, CA, USA), Access AMH (Beckman Coulter, CA, USA), and Elecsys AMH (Roche Diagnostics, Switzerland). AFC was determined using transvaginal ultrasound (5–9 MHz). Differences were assessed 2 × 2 by the Wilcoxon Signed-Rank test, the strength of relationships by the Spearman correlation test, and agreement between assays by the Bland–Altman plots. A $P < 0.05$ was statistically significant.
Main results and the role of chance: Absolute and "per-follicle" AMH levels were, respectively, at 1.97 (0.04–30.66) ng/mL and 0.13 (0.01–0.43) ng/mL/follicle with AMH Gen II ELISA, 1.66 (0.04–30.46) ng/mL and 0.11 (0.01–0.38) ng/mL/follicle with Access AMH, and 1.58 (0.04–26.17) ng/mL and 0.10 (0.01–0.33) ng/mL/follicle with Elecsys AMH. Differences of values between assays were statistically significant ($P < 0.001$). Strengths of correlations between AFC and absolute AMH levels were similar among the 3 assays ($r = 0.83$, $P < 0.001$; $r = 0.83$, $P < 0.001$; and $r = 0.83$, $P < 0.001$, respectively) and Bland–Altman plots revealed adequate agreement between assays.
Limitations, reasons for caution: Our present series was not large enough to determine age-ranked usual values for each assay.
Wider implications of the findings: Automated AMH assays are markedly reliable but show slight yet significant differences (around 5%) in results. Both of them provide results −16% and −20% as low as the manual ELISA assay. Clinicians should be aware of these differences before patient counseling and adaptation of treatments.
Trial registration number: Not Applicable.

P-713 Hormonal parameters before and after withdrawal using vaginal micronized progesterone in anovulatory patients with polycystic ovary syndrome

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Study question: Is the induction of withdrawal bleeding with vaginal micronized progesterone (VMP) necessary for the hormonal evaluation in polycystic ovary syndrome (PCOS).
Summary answer: Induction of withdrawal bleeding with VMP does not significantly alter circulating androgen levels compared to baseline and can be used to assess PCOS patients.
What is known already: One of the diagnostic criteria for PCOS is the baseline increased presence of ovarian androgens. Usually, this assessment is done at the early follicular phase. In amenorrheic patients, it is often done after progesterone withdrawal but it is also performed in amenorrhea. Progesterone administration could suppress ovarian function and androgen synthesis, and it could be a diagnostic bias. Reported data are poor, unclear, with small number of patients assessed and heterogeneous regarding PCOS Rotterdam criteria. In many cases the withdrawal is performed with different kinds of progestins making it hard to compare.
Study design, size, duration: Prospective cohort study including 15 Caucasian reproductive-age patients with PCOS assessed between 2014 and 2015. All subjects fulfilled Rotterdam criteria: chronic ovulatory dysfunction, clinical and/or analytical hyperandrogenemia and polycystic ovaries. Blood sampling was collected at baseline and between the 3rd and the 5th day of withdrawal after administration of VMP 100 mg every 12 h for 7 days.
Participants/materials, setting, methods: Sample 1 (baseline) was obtained the day of the first visit at the clinic for patients with amenorrhea. Sample 2 was obtained between the 3rd and the 5th day of induced withdrawal after 7 days of 100 mg every 12 h vaginal natural micronized P administration. In all subjects, withdrawal bleeding was successful. Blood sample 2 was collected from all patients between 8 and 11 a.m., after an overnight fast.
Main results and the role of chance: 15 patients were included. Two patients presented spontaneous ovulation (confirmed by increased levels of estradiol and P). VMP administration didn't lead to significant modification of hormonal values. There was a trend towards lower levels of gonadotropins during withdrawal bleeding in comparison with baseline values but without significant differences FSH (5.4 ± 1.2 vs. 5.0 ± 1.6, $p = 0.4$) and LH (11.8 ± 7.9 vs. 8.1 ± 4.4 $p = 0.06$).

No significant differences were found between baseline values and values estimated during withdrawal bleeding regarding androgen parameters: free testosterone (5.2 ± 3.2 vs. 4.7 ± 2.7; $p = 0.15$), SHBG (52.3 ± 22.2 vs. 54.3 ± 26.7; $p = 0.50$), DHEAS (2.2 ± 1.9 vs. 2.3 ± 2.1; $p = 0.44$), A4 (2.5 ± 1.0 vs. 2.8 ± 1.3; $p = 0.50$) and 17-OHP (0.6 ± 0.20 vs. 0.7 ± 0.5; $p = 0.86$).

Data from our study indicate that, in women with anovulatory PCOS, the induction of withdrawal bleeding with VMP (7 days of VMP, 100 mg every 12 h) does not significantly modify circulating androgen levels compare to baseline and can be used to time blood sampling in these patients.
Limitations, reasons for caution: Small simple size in this study is a reason of caution. A higher number of patients might have led to different results, although it is hard to find studies with larger sample size about this topic.
Wider implications of the findings: This strategy avoids spontaneous ovulation found in some cases (20% in our group), optimizing schedules and

reaching a diagnosis with the first clinical visit in most of the cases (100% in our group). Therefore it seems to be useful for the assessment of hormonal profile in patients with anovulatory PCOS.
Trial registration number: Non applicable.

P-714 Improved human oocyte *in vitro* maturation in co-culture with cumulus cells from mature oocytes in the same patient: a prospective study

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Study question: Is *in vitro* maturation of immature oocytes (after ovarian hormonal stimulation) improved by co-culture with cumulus cells from mature oocytes in the same patient ?
Summary answer: The co-culture of germinal vesicle (GV) oocytes with cumulus cells from mature oocytes in the same patient improved the success of *in vitro* maturation.
What is known already: Existing evidence shows that cumulus cell contact during oocyte maturation in mice enhances oocyte developmental competence. The oocyte co-culture with cumulus cells during *in vitro* maturation may compensate the suboptimal maturation medium. The *in vitro* maturation of human GV oocytes in co-culture with cumulus cells of the same oocytes has not been proven to be successful due to potential abnormalities in cumulus cells which may disable the oocyte maturation process. Therefore we matured the oocytes *in vitro* in a co-culture with cumulus cells retrieved from mature oocytes in the same patient.
Study design, size, duration: In this prospective 2-year study 174 immature (GV) oocytes from the *in vitro* fertilization programme (IVF) were matured *in vitro*: 79 oocytes in a maturation medium containing FSH and hCG and co-cultured with cumulus cells from the same patient's mature oocytes and 95 oocytes in a conventional way (in the same medium, without co-culture) to compare the maturation rate, chromosomal status and gene expression profile.
Participants/materials, setting, methods: The subgroups of each group of *in vitro* matured oocytes were analyzed on all chromosomes (aCGH) and gene expression profile (microarrays) in comparison to *in vivo* matured oocytes; each group of oocytes was analyzed in three biological replicates consisted of 10 oocytes. The most interesting genes were further validated by qPCR and evaluated using Principal Component Analysis, Student's *T*-test, and one-way ANOVA to elucidate differences between groups; statistical significance was set at $P < 0.05$.
Main results and the role of chance: In co-culture with cumulus cells a higher proportion of oocytes matured *in vitro* than after conventional *in vitro* maturation without co-culture: 77.2% (61/79) vs. 62.1% (59/95), $P < 0.05$, in spite of approximately the same age of donating women (34.5 ± 4.3 vs. 34.1 ± 4.5 years). In oocytes matured *in vitro* in a co-culture there was a tendency to a higher proportion of oocytes which were normal for all chromosomes than in oocytes matured in a conventional way: 43.1% vs. 9.1%. The transcriptome of oocytes matured *in vitro* in co-culture with cumulus cells was shifted toward the *in vivo* matured oocytes in comparison to oocytes conventionally matured *in vitro*, as revealed by principal component analysis. The co-cultured oocytes expressed a significantly lower number of genes that were up-regulated or down-regulated than conventionally matured oocytes in comparison to *in vivo* matured oocytes, including transcription, epigenetics, embryogenesis, and cell cycle-related genes. In general, our study revealed 15 genes which were differently expressed between *in vitro* and *in vivo* matured oocytes and validated by qPCR, including 7 transcription factors. The most prominent, folliculogenesis specific basic helix-loop-helix (FIGLA), is suggested as a biomarker of human oocyte maturation *in vitro*.
Limitations, reasons for caution: The mature oocytes that did not fertilize *in vitro* were considered to be "*in vivo*" matured oocytes and represented a reference (control) group for other groups of *in vitro* matured oocytes. It is possible that they possess some abnormalities because they did not fertilize *in vitro*.
Wider implications of the findings: The oocyte *in vitro* maturation can be improved by co-culture of GV oocytes with cumulus cells retrieved by denudation of mature oocytes in the same patient, a simple principle, which has not been used until now. It may be beneficial to cancer patients to efficiently cryopreserve their oocytes before oncotherapy.
Trial registration number: The study was approved by the Slovenian Medical Ethical Committee.

P-715 A single dose new long-acting GnRH-antagonist Degarelix effectively downregulates LH in stimulated IVF cycles triggered with agonist for oocyte maturation. A proof of concept study

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Study question: To assess whether a new long-acting antagonist degarelix can efficiently suppress LH without negative impact on the pregnancy outcome.
Summary answer: In stimulated IVF cycles, Degarelix compared to daily antagonist regimen, effectively prevents LH surge, with comparable embryo quality, total embryos produced and pregnancy outcome.
What is known already: The use of daily GnRH-antagonist in IVF protocols is well established. A new long acting degarelix was recently licensed for prostate cancer. There is no data for using them in women undergoing IVF.
Study design, size, duration: This prospective case-control study (within same subjects), performed in a private assisted reproduction centre (January to December 2015), enrolled ten healthy women (aged 22–32 years) undergoing ovarian stimulation for oocyte donation programme. All donors underwent 2 consecutive ovarian stimulations with the antagonist protocol, and oocyte maturation triggered with Agonist and oocyte retrieval followed after 36 h. The primary outcomes were incidence of LH surge (>14 mIU/ml) on the day of triggering, total embryos produced and recipient's clinical pregnancy.
Participants/materials, setting, methods: All oocyte donors underwent first, a donation cycle with the classical fixed day−6 antagonist protocol with daily doses of 0.25 mg of ganirelix and 250 IU of recombinant FSH and agonist triggering. After minimum 4 months, underwent a consecutive donation cycle again with antagonist protocol and 250 IU recombinant FSH. However, this second cycle received a single dose of this new agent, degarelix, on day−6. The dose decided to be according to weight at 0.2 mg/kg subcutaneously.
Main results and the role of chance: No LH surge was observed with any of the protocols. However, LH values were significantly lower on the day of triggering in the degarelix group as compared with the daily antagonist group (0.70 vs. 1.98, $p = 0.04$). Importantly, one donor in the degarelix group with LH = 0.1 mIU/ml on the day of agonist triggering had only 5 oocytes retrieved, which was significantly reduced as compared with the first cycle where 17 oocytes had been retrieved. The mean of MII oocytes (9.25 ± 0.7 vs. 10.85. ± 0.8 respectively; NS) and total embryos produced (5.5 ± 0.6 vs. 6.2 ± 0.6 respectively; NS) were comparable between D6-single-dose Degarelix group and the classical D6-daily-antagonist group. Overall, comparable clinical pregnancy rates per embryo transfer were achieved in both groups (66.6% vs. 50.0%, respectively, $p = 0.4$).
Limitations, reasons for caution: Major drawback of this study is the limited number; however, it is a proof of concept with the same donor as control of herself. We attempted to adjust the single dose according to the weight of the patient first and secondly according LH suppression not lasting more than 6 days.
Wider implications of the findings: Based on our results a single dose of the new long-acting GnRH-antagonist Degarelix at a dose of 0.2 mg/kg can efficiently replace repeated daily doses of the already used antagonists. Patient friendly protocols will make IVF easier for patients.
Trial registration number: Non applicable.

P-716 Role of Karyotyping and FMR gene screening in the anticipation of diagnosis of Premature Ovarian Insufficiency (POI)

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Study question: Are their markers in patients in the stage of Transient ovarian failure (TOF) be that can anticipate POI in the future?
Summary answer: Yes, 14% of patients had an abnormal karyotype, 33% had a family history of either primary amenorrhea or POI and 84% had an AMH < 1 μg/ml.
What is known already: 4–18% of the cases who seek clinical evaluation for secondary amenorrhea have POF. TOI is seen 1–2 years before POI and is characterized by irregular menstrual cycles with a raised FSH. One half of patients saw >3 clinicians before they were diagnosed as POI. The X chromosome abnormalities have been largely implicated in literature. ASRM states than 13% of familial and 5% of sporadic cases have a FMR gene pre-mutation.
Study design, size, duration: A case-control study in 2014–2015 of 97 amenorrheic POI patients below 40 years with an FSH >20 IU/l in comparison with 100 control patients >40 years with normal cycles and FSH.
Participants/materials, setting, methods: The patients were recruited from Infertility Institute and Research Center, Hyderabad, India. A detailed clinical and menstrual history was taken from the patients and control patients followed by karyotyping and FMR1 gene screening at the Center for Cellular and Molecular Biology in Hyderabad.
Main results and the role of chance: Of the 97 patients 50% with primary amenorrhea and 14% with secondary amenorrhea had an abnormal karyotype compared to a normal karyotype in all the controls. 81% of the abnormalities were of the X chromosome. None of the patients had an FMR1 gene pre-mutation. The cases and controls had similar CGG repeats in the FMR1 gene. 59% of the patients with secondary amenorrhea were between 25 and 35 years and most with a history of visiting a gynecologist frequently for irregular periods without the anticipated diagnosis of TOF although they had documented high FSH levels. 85% of the patients were seeking infertility treatment, 70% for more than 3 years with a history of menstrual disturbances before the onset of amenorrhea for which they visited a gynecologist. 17 patients had spontaneous pregnancies. All 4 patients with an abnormal karyotype aborted and 8 out of 13 with a normal karyotype aborted. 33% had a family history of either primary amenorrhea or POF. 84% had an AMH < 1 μg/ml and 16% between 1 and 3 μg/ml.
Limitations, reasons for caution: The study sample was small to make any definitive conclusions.
Wider implications of the findings: The study gives us a reason to believe that a simple concentrated history taking and a inclination towards anticipation of an ovarian insufficiency in the future can give the patient a chance to preserve her oocytes or attempt early pregnancy.
Trial registration number: Not required.

P-717 Metformin decreases bone turnover in polycystic ovary syndrome

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Study question: To examine the effect of metformin on bone turnover markers in polycystic ovary syndrome (PCOS).
Summary answer: Three months metformin treatment decreased bone turnover in women with PCOS.
What is known already: The available clinical data in diabetics suggest that metformin may be beneficial for bone metabolism, but prospective randomized trials are needed to demonstrate its possible protective role in PCOS. Pioglitazone treatment in premenopausal women with PCOS has shown to decrease bone mineral density (BMD) and markers of bone resorption.
Study design, size, duration: Spin-off study from a recent randomized, double blind, placebo-controlled study. One hundred and fifty women with PCOS randomized to either metformin (n = 75) or placebo (n = 75) for 12 weeks. Metformin or placebo was started at a dose of 500 mg for the first week and subsequently increased in weekly steps by 500 mg up to 1500 mg in non-obese women and 2000 mg in obese women.
Participants/materials, setting, methods: Concentrations of bone formation marker: procollagen type I amino terminal propeptide (PINP) and bone resorption marker: carboxy-terminal telopeptide of type I collagen (CTX) were analysed at the baseline and after 3 months of metformin/placebo by IDS-iSYS Multi-Discipline Automated Analyzer.
Main results and the role of chance: Compared with the baseline measurements, there were significant decreases in the concentrations of PINP (44.3 ± 22.7 vs. 32.3 ± 13.4 ng/ml, $P < 0.001$) and CTX (0.39 ± 0.19 vs. 0.27 ± 0.14 ng/ml, $P < 0.001$) in the metformin group after 3 months, and this was the case in both obese and non-obese subjects. In the placebo group the levels of PINP (47.5 ± 19.8 vs. 48.1 ± 20.5 ng/ml, $P = 0.712$) and CTX (0.41 ± 0.19 vs. 0.38 ± 0.19 ng/ml, $P = 0.079$) remained unchanged.
Limitations, reasons for caution: Short term study and non-assessment of bone mineral density (BMD) are the most important limitations. Long term studies with BMD measurements and clinical fracture rates are required to demonstrate conclusively the underlying mechanisms in bone remodeling during metformin therapy.
Wider implications of the findings: Reduced bone formation and resorption markers indicate that metformin therapy decreased bone turnover in women with PCOS similarly to that seen with bisphosphonates and selective estrogen receptor modulator treatments. Our findings provide insight into bone remodeling effects of metformin in PCOS.
Trial registration number: NCT00994812.

P-718 Mild versus conventional ovarian stimulation for poor responders undergoing IVF/ICSI: a prospective randomized study

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Study question: Is mild stimulation protocols equal as compared to the conventional GnRH-agonist and antagonist ones in poor responders undergoing IVF/ICSI cycles?
Summary answer: Mild ovarian stimulation is inferior to conventional regimes when applied in poor responders undergoing IVF/ICSI, in numbers of oocytes retrieved, embryos transferred and cancellation rates.
What is known already: Evidence to date indicates that the most efficient approach in managing subfertile poor responders, is the individualization of the treatment protocols, based on antral follicle count (AFC) and anti-Mullerian hormone (AMH) values prior to the IVF cycle, although the success rates remain low. The rationale of the present study lies within the separate benefits of mild stimulation through lower gonadotrophin doses, the lowered cost that accompanies the treatment, together with its suspected equality in terms of effectiveness to the conventionally used protocols for ovarian stimulation.
Study design, size, duration: This RCT was conducted during the period from March 2011 to September 2015. Mild stimulation protocol using clomiphene citrate was compared to the long GnRH-agonist and GnRH antagonist protocols. Randomization was performed by the study coordinator through a phone call to a random non-medical staff belonging to the hospital personnel and by answering to the question "1 or 2" without any further exemplification.
Participants/materials, setting, methods: The study cohort consisted of 58 patients, characterized as "poor responders" according to the Bologna criteria. It was conducted at the Assisted Reproductive Unit of the 3rd Department of Obstetrics and Gynecology, "Attikon" hospital, Medical School, National and Kapodistrian University of Athens.

The primary outcome measure was the number of cumulus oocyte complexes (COCs) retrieved. Secondary outcome measures included duration of stimulation, total dose of gonadotropins administered, clinical pregnancy, miscarriage and live birth per randomised patient.
Main results and the role of chance: Stimulation duration did not differ between groups, as the time of clomiphene citrate use was co-calculated. In contrast, the total gonadotrophin dose was statistically different between groups [1050 (95% CI, 0–2490) vs. 4040 (95% CI, 1800–6795), $p < 0.001$].

A significantly higher number of follicles in the category of 14–15 mm and 18 mm, although not for 16–17 mm, was observed in the control group. Similarly a higher number of COCs and MII oocytes [(1 (95% CI, 0–4) vs. 3 (95% CI, 0–8.4), $p < 0.001$ and 1 (95% CI, 0–4) vs. 2 (95% CI, 0–7.4), $p = 0.001$, respectively], number of fertilized oocytes, cleaved, transferred and frozen embryos (all p values <0.05) was observed in the control group. The endometrial thickness at the day of hCG administration was significantly lower in the study group, compared to the control group [7.8 (95% CI, 4.1–12) vs. 10 (95% CI, 5.1–13.6) mm, $p = 0.015$] and cancellation rates were significantly higher [36.4% (95% CI, 19–53.7) vs. 12% (95% CI, 1.7–25.7), $p = 0.036$].

There was no difference between groups with regards to positive b-hCG [15.2% (95% CI, 2.2–28.1) vs. 20% (95% CI, 3.1–36.9), $p = 0.628$], clinical pregnancy [12.1% (95% CI, 4–23.9) vs. 20% (95% CI, 3.1–36.9), $p = 0.412$], and live birth [9.1% (95% CI, 1.3–19.4) vs. 12% (95% CI, 1.7–25.7), $p = 0.719$] rates, neither to miscarriages [40% (95% CI, 28–100) vs. 40% (95% CI, 28–100), $p = 1.000$].

Limitations, reasons for caution: Limitations of the study would be the lowered clinical value of the results attributed to the reduced cohort size, the existence of two conventional protocols, representing the control group and the difference in terms of oocyte quality and quantity when stimulated between poor responders at a young and advanced age.

Wider implications of the findings: There is a varying discrepancy on the outcomes reported in this study with all four trials published to date, using the same regime and applying it to poor responders. The importance of the reported results lies in the fact that poor responders have an anticipated poor outcome in conventional IVF protocols.

Trial registration number: NCT01319708.

P-719 Anti-Müllerian Hormone (AMH) affects Forkhead box L2 (FOXL2) expression: possible implications in early phases of folliculogenesis

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Study question: The objective was to analyze the inhibitory effect of Anti-Müllerian hormone (AMH) on steroidogenesis through a possible modulating effect on the expression of Forkhead box L2 (FOXL2) transcription factor.

Summary answer: recombinant human AMH (rhAMH) increased the expression of FOXL2 in primary cultures of human granulosa cells (hGCs) *in vitro*.

What is known already: *AMH* and *FOXL2* are two pivotal genes expressed in hGCs, and both exert a similar inhibitory function on activation and follicular growth, in order to preserve the ovarian follicle reserve.

Notably both genes contribute to inhibit steroidogenesis decreasing or preventing the activation of gonadotropin-dependent aromatase *CYP19A1*. Although FOXL2 among others is considered a transcriptional activator of the *AMH* gene, other studies showed that AMH induces *FOXL2* expression in rodent models, suggesting that *FOXL2* is an endogenous downstream target of AMH.

Study design, size, duration: *FOXL2* gene expression elicited by rhAMH was evaluated by dose response (1, 10, 50, 100 ng/ml of rhAMH) and time-course (1, 3, 6, 24 h incubation with rhAMH) studies in hGCs primary cultures. Untreated hGCs were used as control. The expression of *CYP19A1* aromatase and the concentration of intracellular adenosine-3',5'-cyclic monophosphate (cAMP) were evaluated as positive control in order to test the efficiency of rhAMH treatments.

Participants/materials, setting, methods: Primary hGCs cultures were rhAMH treated *in vitro*. Negative controls using corresponding amount of AMH vehicle were performed. Intracellular content of cAMP was measured by immuno-enzymatic reaction. Total RNA or proteins were purified and quantified. The *CYP19A1* and *FOXL2* gene expression, normalized by housekeeping *ribosomal protein subunit 7* (*RpS7*) gene, was evaluated by RT-qPCR. Each reaction was repeated in triplicate. Statistical analysis was performed. Extracted proteins underwent Western-blot analysis with anti-FOXL2 and anti-β-tubulin antibodies.

Main results and the role of chance: rhAMH treatments reduced the content of intracellular cAMP of hGCS as expected. To understand if different concentrations of rhAMH at different time points could affect the *FOXL2* gene expression, samples were analyzed by RT-qPCR. Although the majority of the treatments tested did not produced significant differences in *FOXL2* activation compared to the control, different dosages of rhAMH (10, 50, 100 ng/ml) after 3 h of incubation, increased the *FOXL2* gene expression. However due to statistical analysis, only the 50 ng/ml treatment shows a consistent significant activation of *FOXL2* gene expression. Finally, based on RT-qPCR data previously obtained, the effect of 3 h incubation with 50 ng/ml rhAMH on FOXL2 protein production in hGCs primary cultures was analyzed by Western-blot. The semi-quantified amount of FOXL2 protein normalized by β-tubulin generated by rhAMH treatment is significantly augmented in comparison to untreated controls.

Limitations, reasons for caution: This study was conducted only *in vitro* on primary hGCs culture recovered from patients who had undergone IVF protocol. Even though a general positive effect in the modulation of *FOXL2* by rhAMH is recognizable in most of the treatments tested, a high individual variability, although statistically not significant, was observed.

Wider implications of the findings: This study shows the existence of common pathways between AMH and FOXL2 in hGCs. AMH could be able to regulate folliculogenesis by modulating both gene and protein expression levels of FOXL2. Because FOXL2 induces AMH transcription, these ovarian factors can be finely regulated by a positive feedback loop mechanism.

Trial registration number: NO.

P-720 Luteal phase ovarian stimulation produces competent oocytes in women with diminished ovarian reserve

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Study question: Do oocytes retrieved from follicular phase (FP) and luteal phases (LP) of the same cycle in patients with diminished ovarian reserve (DOR) have similar competency?

Summary answer: Oocytes retrieved from FP and LP in the same IVF cycle have comparable fertilization rate and form embryos with comparable cleavage and blastulation rates.

What is known already: Folliculogenesis occurs in a wave-like fashion indicating that there are multiple follicular recruitment waves in the same menstrual cycle. Patients with DOR often produce poor quality and quantity oocytes by IVF.

Study design, size, duration: In order to increase the number of oocytes collected in the same IVF cycle, double ovarian stimulation in the FP and LP were performed. From July 2013 to December 2014, 60 patients with DOR defined by Bologna criteria, aged between 28 and 48, had 87 IVF cycles.

Participants/materials, setting, methods: Patients were either stimulated with minimal stimulation protocol (MSP) or monitored in natural cycle in FP. If 2 or more small follicles (≤10 mm) were available after oocyte retrieval, they were stimulated with MSP in the LP. In both FP and LP, oocytes were retrieved when the leading follicle reached a size of at least 18 mm. The number of oocytes recovered, number of metaphase II (MII) oocytes, fertilization, cleavage and blastulation rates were assessed.

Main results and the role of chance: Mean age and BMI of the participants were 42.43 ± 3.71 years and 22.75 ± 3.78 kg/m^2, respectively. The average interval of days between oocyte retrieval during FP and LP was 9.44 ± 3.23. Of the 87 IVF cycles studied, 125 follicles in FP and 151 follicles in LP were retrieved. The oocyte retrieval rate was significantly lower in LP (68.21%) compared to that in FP (84%) ($p = 0.002$), but there was no difference in the percentage of MII oocytes (58.94% in LP vs. 62.4% in FP, $p = 0.34$), no difference in fertilization rate (78.67% in LP vs. 86.58% in FP, $p = 0.19$) or blastomere number (7.10 ± 1.90 in LP vs. 7.37 ± 2.19 in FP, $p = 0.47$) on day 3 of culture. 20 of 49 embryos (40.82%) from FP and 24 of 48 embryos (50.00%) from LP reached the blastocyst stage ($p = 0.36$). Of 31 blastocysts that were tested with preimplantation genetic screening, 2 of 13 from FP and 1 of 18 from LP were euploid.

Limitations, reasons for caution: The study may be underpowered to detect statistical significance. The data on comparison of implantation and pregnancy outcomes between FP and LP ovarian stimulation protocols is under collection.

Wider implications of the findings: Oocyte retrieval from both follicular phase and luteal phase by double stimulation in the same cycle provides a new

and safe approach in increasing the amount of oocyte retrieved in the same IVF cycle for the patients with DOR.
Trial registration number: N/A.

P-721 Is there a bridge between diminished ovarian reserve and inflammation? A pilot study

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Study question: Can diminished ovarian reserve (DOR) be linked to immunologic bias?
Summary answer: Immune mediators expressed by cumulus cells are significantly distinct in DOR women vs. control, and specifically correlated with ovarian reserve markers.
What is known already: Diminishment of ovarian reserve (early or not) is recognized as a limiting factor in Assisted Reproductive Technology (ART). Throughout the growth of the ovarian follicle, the oocyte is in close contact with neighboring cells such as cumulus cells (CC), which contribute to the oocyte maturation and quality. Thereby, the oocyte-cumulus dialogue is representative of the quality of the oocyte, which in turn is indicative of the embryo quality and a crucial point in ART. However, the inflammatory environment in which the oocyte evolves is not clearly established, and it remains unclear how ovarian inflammatory background can impact the ovarian reserve.
Study design, size, duration: Retrospective clinical study in which the immune gene expression in CC associated with mature oocytes in DOR and control patients were investigated.
Inclusion criteria: standard IVF and CC from mature oocyte.
Exclusion criteria: Polycystic ovary syndrome, autoimmune diseases.
Participants/materials, setting, methods: 47 CC samples from 8 women (4 DOR, 4 controls) who underwent IVF protocol at Clinique Ovo and provided a written informed consent to participate in a research protocol approved by the ethics committee, were collected. For each sample, mRNA was extracted and subsequently, quantitative real-time PCR was performed. Statistical analyses were performed using Student's *t*-Test and Pearson correlation test.
Main results and the role of chance: In this pilot study, we report that DOR affects the expression of interleukin (IL) 1 members in CC compared with control. Independently of age, mRNA expression of lL1R1 was significantly increased in DOR ($p = 0.0110$). This increase was significantly and negatively associated with AMH (serum level) and antral follicle count (AFC) ($p = 0.0180$ and $p = 0.0001$ respectively). Interestingly, IL1B was significantly and negatively correlated with AMH serum level ($p = 0.0137$). In turn, IL1RN (IL1 antagonist receptor) was significantly increased in DOR ($p = 0.0099$) and negatively correlated with AMH ($p = 0.0182$) and AFC ($p = 0.0001$). Despite IL1RN increase, IL1B/IL1R1 balance was still functional and positively correlated with FSH serum level ($p = 0.0147$ and $p = 0.003$). We observed that, the percentage of circulating neutrophil was significantly increased in DOR and was associated with AMH decrease. Curiously, the major neutrophil chemotactic factor, IL8, was significantly increased in DOR ($p = 0.0001$) and negatively correlated with AMH ($p = 0.0135$) and AFC ($p = 0.0001$). It is important to note that, IL8 and IL1RN mRNA were positively associated with age ($p = 0.0105$ and $p = 0.0001$, respectively). These results indicate that IL1B–IL1R1 axis over expression is correlated with DOR process, and IL8 and IL1RN over expression is correlated with DOR and ovarian ageing process, which can be linked to blood neutrophil count.
Limitations, reasons for caution: We presented a pilot study which needs further investigation. Due to low sample material, only mRNA expression can be assessed.
Wider implications of the findings: Identifying ovarian immune bias as responsible for the DOR would be a new way to manage infertility. To the best of our knowledge, it is the first time that immune mediator expression by CC is linked with DOR, ageing and neutrophil, has been reported.
Trial registration number: NA.

P-722 High AMH doesn't guarantee high pregnancy rate in old patients: AMH should be interpreted with age for outcome prediction of IUI or IVF

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Study question: Is pregnancy rate in old patients who have high AMH levels comparable with that in young patients after IUI or IVF?
Summary answer: AMH should be interpreted with age, because IUI or IVF pregnancy rates in old patients with high AMH are lower than those in young patients.
What is known already: AMH is related with ovarian function. AMH is higher in younger patients and higher levels of AMH corresponded to higher pregnancy rates. However, some young patients have lower AMH levels than average for their age and some old patients have high AMH levels. Whether certain AMH level in old patients can be interpreted the same as the similar AMH level in young patients in is yet to be elucidated.
Study design, size, duration: In this retrospective cross sectional study IUI cycles from January 2014 to July 2015 and IVF cycles from January 2015 to August 2015 were reviewed. Data were collected when their AMH levels were available within a year from IUI or ovum pick-up day.
Participants/materials, setting, methods: 1,044 cycles of IUI and 952 IVF cycles were reviewed. Patients were divided into 2 age groups; 1) young age group (age < 40, $n = 1.549$), 2) old age group (age 40 or more, $n = 447$). AMH percentiles were calculated in each age group and pregnancy rate were estimated depending on age-adjusted AMH percentiles.
Main results and the role of chance: Subgroups were defined depending on AMH percentiles in each age group and AMH cut off values were as follows; 0.73, 0.10 for 5 percentile, 1.20, 0.30 for 10 percentile, 2.27, 0.60 for 25 percentile, 3.91, 1.39 for 50 percentile, 6.56, 2.85 for 75 percentile, 9.79, 5.78 for 90 percentile, 13.41, 8.35 for 95 percentile in young and old age group, respectively. Pregnancy rate of IUI in less than 5 percentile AMH percentile subgroup in young patients was lower (7.1%) than those in other subgroups (23.7%, 16.1%, 12.9%, 17.9%, 19.7%, 27.5% and 26.8% for 10, 25, 50, 75, 90, 95, 95 percentiles or more, respectively). Pregnancy rates of IVF were similar between subgroups in young patients (34.5%, 36.6%, 37.5%, 46.4%, 49.5%, 45.6%, 35.0% and 41.7% in ascending order). In old patients pregnancy rate after IUI were similar between subgroups (0%, 0%, 8.3%, 4.3%, 16.0%, 0%, 0% and 0% in ascending order). While IVF success rates were higher in more than 50 percentile subgroups (18.4%, 18.1%, 20.8%, 29.4% and 66.7% for 50, 75, 90, 95, more than 95 percentile subgroups, respectively) than those in lower AMH percentile subgroups (0%, 0% and 10.9% for 5, 10, 25 percentile subgroups, respectively).
Limitations, reasons for caution: AMH percentile was calculated in study population. Age-adjusted AMH percentile cut off might be changed when investigated in larger group. Further study is necessary to extrapolate this result to general infertile population.
Wider implications of the findings: Pregnancy rate of IUI is low in young patients with low AMH while IVF success rate is maintained. In old patients pregnancy rate of IUI is low regardless of their AMH. Old patients with high AMH level might have benefit from IVF because pregnancy rate is much higher than IUI.
Trial registration number: This is retrospective study.

P-723 Effect of oral contraceptives on change in endocrine and metabolic profiles of women with polycystic ovary syndrome; 1-year follow-up preliminary cohort study

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Study question: To evaluate the effects of oral contraceptives (OC) on change in endocrine and metabolic profile of women with polycystic ovary syndrome (PCOS) for 1 year.
Summary answer: Long term use of OC could change endocrine and metabolic profile of women with PCOS.
What is known already: PCOS is a common reproductive endocrine disease that is seen among women of reproductive age. OC is commonly used to manage the symptoms of menstrual irregularity and a- or oligo-menorrhea. Although this medication have been beneficial in treating PCOS symptoms, its effect on endocrine and metabolic profile with long time follow-up is still not entirely elucidated.

Study design, size, duration: Fifty-two of women with PCOS diagnosed by Rotterdam criteria were recruited to check endocrine and metabolic profile with annual follow-up in this preliminary cohort study, and there was no attrition.
Participants/materials, setting, methods: Before and after 1-year OC use, endocrine and metabolic profile as the followings were checked in women with PCOS; BMI, basal and PP2 insulin, basal and PP2 glucose, HOMA-IR, cholesterol, triglyceride, HDL, LDL, HbA1c, CRP, estradiol, FSH, LH, AMH, Testosterone, SHBG, free androgen index (FAI), DHEAs, 17-α-OHP, TSH, prolactin. Data were analyzed by Paired *t*-test and McNemar's test where appropriate.
Main results and the role of chance: Among endocrine profiles, basal insulin (10.5 μIU/mL vs. 12.8 μIU/mL, $P = 0.017$), SHBG (63.1 nmol/L vs. 154.9 nmol/L, $P < 0.001$) and DHEAs (185.3 μg/dL vs. 220.5 μg/dL, $P = 0.011$) were increased, and testosterone (1.2 ng/mL vs. 0.7 ng/mL, $P < 0.001$), 17-α-OHP (2.4 ng/mL vs. 1.1 ng/mL, $P = 0.002$) and AMH (15.6 ng/mL vs. 11.6 ng/mL, $P = 0.003$) were decreased after 1-year OC use. In metabolic profiles, triglyceride (83.2 mg/dL vs. 124.0 mg/dL, $P = 0.015$) was increased with 1-year OC use. HOMA-IR was similar although FAI (9.8 vs. 4.7, $P < 0.001$) was decreased by 1-year OC use. Before and after 1-year OC use, women with abnormal HOMA-IR (>2) were 40.0% and 64.4% ($P = 0.901$), and women with abnormal FAI (>4) were 73.3% and 37.8% ($P = 0.487$).
Limitations, reasons for caution: Further study should be necessary with larger sample size and longer follow-up over 5-year.
Wider implications of the findings: One-year OC use could change hormonal status and lipid profiles although it could not change proportion of women with androgen excess significantly. Effect of longer OC use over 1 year should be evaluated in further study.
Trial registration number: 2012-11-0022 (KUGH13147-004).

P-724 Assessment of the Access AMH assay as an automated, high-performance replacement for the AMH Generation II manual ELISA

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Study question: To evaluate the performance of the Access Anti Müllerian Hormone (AMH) assay and directly compare it to the modified Generation II (Gen II) ELISA method.
Summary answer: The Access AMH automated assay exhibits high levels of stability and sensitivity, and results correlate with the existing ELISA method and between analyser platforms.
What is known already: A number of AMH immunoassays have been developed in the past; however, a lack of standardisation and technical issues has led to confusion in interpretation of results and scepticism of AMH test reliability. The manual Gen II ELISA method used to measure AMH from Beckman Coulter has recently been superseded by a fully automated AMH immunoassay for both the Access2 and DxI800 instruments. Studies have revealed good correlation between the Gen II and Roche Elecsys AMH assays; however, consensus on correlation between the Gen II ELISA and the new Access AMH assay has not been reached.
Study design, size, duration: The precision, stability, linearity, measurement range and detection limits were established and a correlation study was performed ($n = 142$) to compare the Access AMH assay to the ELISA method using Passing–Bablok and Bland–Altman methods of comparison. A double blinded retrospective observational study was conducted ($n = 492$) to verify that a fertile age-related AMH range previously established using the Gen II ELISA could be used to interpret results from the new automated Access assay.
Participants/materials, setting, methods: The Beckman Coulter Access AMH immunoassay was assessed for use on the Access2 and DxI800 analysers using de-identified patient sera and quality control or calibrator material consisting of human recombinant AMH. The fertile AMH reference range was conducted on 492 pregnant women in their first trimester, aged between 20 and 44 years, who had all conceived spontaneously without the use of ovarian stimulation drugs within 2–3 months of attempted conception.
Main results and the role of chance: The Access AMH assay showed good performance across the measuring range for both intra-assay (CV 1.41–3.30%) and inter-assay (CV 3.04–5.76%) precision and acceptable sample stability. The assay was linear over the range of values recommended by the manufacturer, allowing for accurate reporting within the reported range. The two assay types correlated tightly ($R^2 = 0.9822$ and 0.9832 for Access2 and DxI800), and differences observed between the Access2 and DxI800 analysers were within clinically acceptable ranges, indicating that the methods are interchangeable. Furthermore, we demonstrated that results from a published fertile reference range for the Gen II ELISA correlate with those from the automated Access AMH assay (CV 6.7%).
Limitations, reasons for caution: Samples for the correlation studies required freeze/thawing between testing which may have been a source of sample bias. Limitations of this study include that the reference range is only representative of women with proven fertility and is only applicable to the Beckman Coulter AMH Gen II ELISA and Access assays.
Wider implications of the findings: This study verified the published performance of the Access AMH assay and demonstrates that results from the preceding Gen II ELISA are interchangeable with the new automated Access assay. These findings are an encouraging step towards the necessary establishment of universal cut-off values and the standardisation of AMH assay results.
Trial registration number: None.

P-725 Clomiphene, metformin, letrozole, tamoxifen or combined clomiphene-metformin for polycystic ovary syndrome – a systematic review and individual participant data network meta-analysis

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Study question: Which is the most effective pharmacologic ovulation induction therapy for women with polycystic ovary syndrome (PCOS)?
Summary answer: In treatment-naïve women with PCOS, the relative effectiveness of letrozole over other drugs is the highest in women with a high BMI.
What is known already: In women with anovulatory infertility PCOS, Clomiphene has been considered as the first-line pharmacologic therapy for ovulation induction for decades. Clinical decision making based on pairwise meta-analyses does often not reflect the whole picture. A recent network meta-analysis on WHO group II anovulation concluded that letrozole and combined clomiphene–metformin are probably superior to other treatments, including clomiphene alone. However, several included studies in this network meta-analysis also contain women with ovulation induction history.
Study design, size, duration: We performed an individual participant data (IPD) network meta-analysis. We searched MEDLINE, EMBASE and Cochrane Central Register of Controlled Trials databases up to October 2015 without language restrictions and conducted a systematic review and IPD network meta-analysis on relevant randomised controlled trials (RCTs). The primary outcome was clinical pregnancy rate, with live birth, miscarriage, and multiple pregnancy rates as secondary outcomes.
Participants/materials, setting, methods: We included RCTs comparing at least two of following interventions in treatment-naïve women with PCOS: clomiphene, metformin, combined clomiphene–metformin, letrozole and tamoxifen. Studies were excluded if they did not at least partly include treatment-naïve women. First, we performed an aggregate network meta-analysis using a random-effects multiple regression model in STATA. For studies also including treatment-experienced women, only IPD of treatment-naïve women was used. We then performed subgroup analyses using IPD.

Main results and the role of chance: For the aggregate network meta-analysis, we detected 21 trials comprising of 2,500 treatment-naïve women with PCOS across five interventions. Compared to clomiphene, letrozole (odds ratio [OR] 1.79, 95% confidence interval [CI] 1.18–2.70) and combined clomiphene–metformin (OR 1.67, 95% CI 1.03–2.70) resulted in statistically significant higher pregnancy rates. IPD of 1,291 women with PCOS from eight trials in the USA, The Netherlands, New Zealand, India and Italy were provided by authors. Subgroup analyses on body mass index (BMI) showed that only in the highest BMI tertile (BMI > 32.5), letrozole led to higher clinical pregnancy rate than clomiphene (OR 2.88, 95% CI 1.04–7.93) and metformin (OR 14.85, 95% CI 2.47–89.27) alone. In women with a BMI ≤ 32.5, there were no differences between interventions in any of the subgroups.
Limitations, reasons for caution: Only 52% IPD of all participants could be used for this abstract. Since several other authors have agreed to share their IPD, this proportion is expected to increase. Additionally, the included studies are clinically heterogeneous in many aspects including diagnostic criteria, patient characteristics, dosage and duration of the interventions.
Wider implications of the findings: Two major issues limited the application of several major multi-centre RCTs on ovulation induction: 1) not all women were treatment-naïve; 2) not all interventions were compared directly. The current IPD network meta-analysis addressed these problems and updates the current evidence. However, long-term follow-up is still urgent for pharmacologic ovulation induction.
Trial registration number: None.

P-726 Gonadotropin-releasing hormone agonist versus hCG for triggering of final follicular maturation in GnRH antagonist protocol – differential effects on day 3 embryo quality

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Study question: Does triggering of final follicular maturation with Gonadotropin-releasing hormone (GnRH) agonist instead of hCG in GnRH antagonist protocol improve the quality of Day 3 embryos?
Summary answer: The percentage of Grade I embryos on Day 3 is significantly higher in patients triggered with GnRH agonist vs. hCG in GnRH antagonist protocol.
What is known already: The introduction of GnRH antagonists in controlled ovarian hyperstimulation protocols has brought a renewed interest in the use of GnRH agonist to trigger final follicular maturation, causing a surge of both endogenous FSH and LH, mimicking the natural cycle surges and therefore considered to be more "physiologic." There is an extensive body of evidence demonstrating that triggering with GnRH agonist (vs. hCG) had no differences in the number of oocytes retrieved, percentage of MII oocytes and fertilization rates, whereas it seems that can almost eliminate ovarian hyperstimulation syndrome (OHSS).
Study design, size, duration: In the present prospective cohort study were included 44 consecutive subjects at risk for OHSS undergoing *in vitro* fertilization (IVF) treatment cycles, between January 2014 and December 2015.
Participants/materials, setting, methods: Controlled ovarian stimulation was applied with GnRH antagonist protocol. The study group (21 women) received for triggering a single bolus of GnRH agonist (0.2 mg) vs. rhCG (250 μg) the control group (23 women). Decision of triggering was based on the estradiol levels and the number of developed follicles. Oocyte retrieval was performed 34–36 h later, followed by simple IVF or ICSI. Embryos were cultured until day 3 and graded according to the established criteria.
Main results and the role of chance: No significant differences were observed between patients triggered by GnRH agonist vs. hCG in terms of age (35.05 ± 4.65 vs. 35.39 ± 4.63, respectively) and FSH levels (6.08 ± 1.41 mIU/mL vs. 5.89 ± 0.88 mIU/mL, respectively). In the group triggered with GnRH agonist, the average estradiol levels on the day of ovulation triggering was significantly higher compared to the group triggered with hCG (6173.86 ± 1513.417 pg/mL vs. 4132.00 ± 1541.18 pg/mL, respectively, $p < 0.01$). No significant differences were seen between the two groups in terms of the number of oocytes retrieved (22.52 ± 8.24 vs. 21.96 ± 3.07, respectively) as well as the number of MII oocytes (18.24 ± 6.94 vs. 17.09 ± 4.13, respectively). Regarding embryo quality on Day 3, percentage of grade I embryos was significantly higher in the study group (GnRH agonist trigger) compared to that in the control group (hCG trigger) (69.48% vs. 56.88%, respectively, $p < 0.01$); percentage of grade II+III embryos was significantly lower in the study group (GnRH agonist trigger) compared to the control group (hCG trigger) (30.15% vs. 42.38%, respectively, $p < 0.01$). Interestingly, no differences in pregnancy rates were seen between the two groups (56% vs. 65%, respectively).
Limitations, reasons for caution: A limitation of our study is its small number of cases. Some caution is also warranted in interpreting the pregnancy rates due to the fact that all the women triggered with GnRH agonist (GnRHa) had frozen–thawed embryo transfer whereas women triggered with hCG had either fresh or frozen–thawed embryo transfer.
Wider implications of the findings: Studies that compared the effect of GnRHa trigger vs. hCG on the number of top-quality embryos concluded that they are either comparable or in favor of GnRHa trigger. Following our observations, in patients at risk to develop OHSS, GnRHa improves embryo quality on Day 3 resulting in improved reproductive outcome.
Trial registration number: Not applicable.

P-727 Follicular flushing in natural cycle IVF neither affects the length of the luteal phase nor the luteal body hormone production – a prospective controlled study

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Study question: Does follicular flushing in Natural Cycle IVF (NC-IVF) affect the length of the luteal phase and the luteal phase concentrations of progesterone and 17beta-estradiol?
Summary answer: Follicular flushing in NC-IVF does affect neither the length of the luteal phase nor the luteal phase concentrations of progesterone and estradiol.
What is known already: In contrast to multifollicular IVF, follicular flushing increases the efficacy of monofollicular IVF such as NC-IVF by higher oocyte yield and higher transfer rates according to two retrospective studies. However, as follicular flushing causes loss of granulosa cells, follicular flushing might negatively affect the formation and endocrine function of the luteal body, requiring luteal phase support with progesterone and possibly also estrogen.
Study design, size, duration: A prospective cohort phase II study was performed with 24 women undergoing NC-IVF in 2013 and 2015. Follicle flushing was expected to reduce luteal phase length (primary outcome) and decrease luteal phase progesterone and estradiol concentrations (secondary outcome). 22 women were required to detect a shortening of the luteal phase due to flushing of the follicles. Data were analysed using a paired Wilcoxon signed rank test with continuity correction for each time point separately.
Participants/materials, setting, methods: Women (age 18–40 years) with regular menstrual cycles (26–32 days) and AMH-concentrations >5 pmol/L were screened at a University based infertility center. Women first underwent a training cycle with HCG induced ovulation, followed by the analysis of the length of luteal phase and concentrations of progesterone and estradiol on day 2–3, 6–7 and 10–11 post ovulation. A second (NC-IVF) flushing cycle was identically performed but follicles were aspirated and flushed 3–5 times.
Main results and the role of chance: 49 women were screened, 46 were enrolled and 24 women finalized the study. The high drop out rate of 52% was due to 11/46 women who became pregnant during the first cycle and 11/46 women who preliminarily stopped the study due to other reasons. Data of luteal phase length was complete in 23/24 women. In 7 of these women luteal phase was shorter (29.2%), in 4 women luteal phase length was equal (16.7%) and in 12 women luteal phase was longer (50.0%) following flushing of the follicles. Overall, the difference in luteal phase length was not significant (median duration [interquartile range] in training cycle: 13 [12; 14.5], flushing cycle: 14 [12.5; 14.5], median difference (95% CI): 0.5 (−0.5 to 1.5); paired Wilcoxon signed rank test: $V = 116.5$, $p = 0.391$). Median progesterone and estradiol concentrations did not differ significantly in the flushing cycle compared to the training cycle (median difference (95% CI) in progesterone [pmol/L], early in cycle: −5.2 (−11.2 to 1.3); mid: 1.1 (−6.6 to 8.7); late: −1.2 (−10.2 to 6.1); median difference (95% CI) in estradiol [pmol/L], early: 65.0 (−3.5 to 172.5); mid: 31.1 (−104.5 to 199.0); late: −17.2 (−135.0 to 85.5); all $p > 0.05$).

Limitations, reasons for caution: The study was not designed to analyse the impact of follicle flushing on pregnancy rates. The conclusion that women might not need luteal phase support in NC-IVF is only based on the study parameters but not on pregnancy rates.
Wider implications of the findings: NC-IVF is favoured by many women due to lower treatment induced psychological stress and lower costs. The result of the study suggest that luteal phase support is not required in NC-IVF, even if the follicles are flushed, thereby allowing further treatment simplification by avoiding uncomfortable luteal phase support.
Trial registration number: KEK-BE 206/12

P-728 Identification of differentially expressed long non-coding RNAs in follicular granulosa cells from polycystic ovary syndrome patients and controls

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Study question: What roles of LncRNAs in pathology of PCOS?
Summary answer: There are some differentially expressed LncRNAs between PCOS patients and controls.
What is known already: Long non-coding RNAs (lncRNAs) are molecules longer than 200 nucleotides with non-protein coding transcripts. Until now, a number of lncRNAs have been identified. Many lncRNAs have significant impact on transcriptional and translational output and many studied have shown that lncRNAs play a key role in regulating diverse cellular processes, which contain intracellular trafficking, chromatin remodeling, transcription and post-transcriptional processing.
Study design, size, duration: PCOS patients in IVF/ICSI were referred from the reproductive medicine center at Ninth Hospital affiliated with Shanghai Jiao Tong University from January 2013 to December 2015.
Participants/materials, setting, methods: The follicular granulosa cells used in this study were obtained from patients and controls. Total RNA was extracted by using the AllPrep DNA/RNA/miRNA Universal kit. The fluorescence labeled cRNA targets for the Agilent Human lncRNA 4 × 180 K. KGN cells were transfected either lncRNA mimics or their controls to KGN cells with HiPerFect transfection reagent. The supernatant was measured for concentrations of estradiol with the UniCel DxI 800 immunoassay system.
Main results and the role of chance: 63,431 lncRNAs were examined in this study. A total of 1,154 lncRNAs and 853 mRNAs were identified to be significantly altered in 3 pairs of PCOS granulosa cells and controls (fold change > 2; $p < 0.05$). Of these differently expression lncRNAs, 305 lncRNAs were up-regulated and 849 were down-regulated. Out of the down-regulated lncRNAs group, lncRNA CUST_12429 has the greatest degree of down-regulation (fold change = 68.27807); and in the up-regulation group, lncRNA CUST_34147 has the greatest degree of up-regulation (fold change = 0.063102). We validated some of differentially expressed lncRNAs. We then transfected lncRNA mimics and corresponding controls into the KGN cell line. We found that lncRNA CUST_12429 regulated estradiol secretion.
Limitations, reasons for caution: Exact mechanism of LncRNAs in PCOS should be explored further in the future. Target genes and pathways should also be explored.
Wider implications of the findings: This study identified a number of LncRNAs in granulosal cells and laid a foundation for investigating roles of LncRNAs in pathology of PCOS.
Trial registration number: Not required.

P-729 Impact of polymorphisms of gonadotropins and their receptors on controlled ovarian stimulation: a prospective observational study

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Study question: Which effect do polymorphisms of gonadotropins and their receptors have on stimulation outcomes in IVF patients co-treated with a GnRHa long down-regulation protocol?
Summary answer: Allele C of FSHR-29, LHCGR-291 and FSHR-680 all resulted in a significantly increased cumulative *r*-FSH dose: total number of oocytes or mature oocytes ratio.
What is known already: Specific polymorphisms might influence controlled ovarian stimulation in women undergoing IVF/ICSI. Data regarding the possible interactions of these polymorphisms are still scanty, especially as regards LHCG-R polymorphisms.
Study design, size, duration: Prospective observational study in 100 normogonadotropic IVF/ICSI patients came from three public IVF Units.
Participants/materials, setting, methods: Normogonadotropic Caucasian women fulfilling the following inclusion criteria were enrolled: age 20–34 years; BMI 20–27 kg/m2; basal FSH ≤ 10 IU/l; functional ovaries. Exclusion criteria were: uterine anomalies; endocrine, genetic or immunological disorders; PCOS; history of impaired ovarian response (≤ 4 oocytes retrieved) in at least one IVF/ICSI cycle. Patients underwent a GnRH long down-regulation protocol with a starting dose of 150 IU of recombinant FSH daily. Six polymorphisms were genotyped.
Main results and the role of chance: The following polymorphisms were analyzed: FSHR-680 (rs6166); FSHR-min29 (rs1394205); LHCGR intronic (rs4073366); LHCGR-291 (rs 12470652); LHCGR-312 (rs2293275); FSHβ-2623 (rs6169).

Basal FSH levels were significantly lower in homozygotic carriers of FSHR-630 (T/T) than in heterozygotic C/T ($p = 0.023$). Lower basal estradiol levels were seen in homozygotic carriers of FSHR-29 promoter C/C compared to heterozygotic C/T ($p = 0.045$). Basal estradiol levels and number of fertilized and mature oocytes were lower in homozygotic carriers of LHCGR-291 (T/T) compared to heterozygotic C/T ($p = 0.035$ and $p = 0.05$ respectively). The presence of allele C on both FSHR-min29 and LHCGR-291 caused an increased ratio between the cumulative r-FSH consumption and the total number of oocytes as well as mature oocytes (RR: 5.47, CI 95%: 3.13–7.81, $p < 0.001$). This observation was also confirmed when polymorphisms of FSHR-680 were included in the analysis. Specifically, the presence of allele C on these three genes was related to an increased ratio between the cumulative FSH consumption and the total number of oocytes or mature oocytes (RR: 5.44, CI 95%: 3.18–7.71, $p < 0.001$).
Limitations, reasons for caution: Although limited by the small size of the population, these findings confirm a possible interaction between multiple polymorphisms in assisted reproductive technology.
Wider implications of the findings: These data support the concept that the ovarian response to exogenous FSH seems to be determined by the interaction of specific genetic traits. Moreover, this study shows an involvement of the LHCGR-291 polymorphism in ovarian response to exogenous gonadotropins.
Trial registration number: Not applicable.

P-730 Outcome after non-hCG triggered serum-free IVM in patients with PCOS: a freeze-or-fresh transfer strategy based on number of available embryos

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Study question: What is the outcome of IVM using a strategy of fresh blastocyst transfer or freeze-all d3/frozen embryo transfer (FET), depending on the number of available embryos?
Summary answer: After non-hCG triggered IVM, a strategy of fresh blastocyst transfer or freeze-all d3/FET results in equivalent cumulative ongoing pregnancy rates (OPR).

What is known already: After minimal-stimulation, non-hCG triggered IVM and blastocyst transfer, clinical pregnancy rates of >40% per cycle have been reported in patients with PCOS. However, blastocyst yield after IVM is lower than after IVF. When IVM embryos are transferred freshly at cleavage stage, reported clinical outcomes are dramatically low but they can be significantly improved using a freeze-all strategy, followed by FET in an artificial cycle. The clinical potential of a dual strategy combining high pregnancy rates and low cancellation rates, based on the available number of good-quality d3-embryos after IVM, is currently unknown.
Study design, size, duration: This study is a cohort analysis of retrospective data from the first IVM cycle of 195 Rotterdam-PCOS patients treated at a tertiary university based infertility centre between April 2014 and October 2015.
Participants/materials, setting, methods: Consecutive patients between 20 and 41 years underwent non-hCG triggered IVM (30 h) after a short course of HP-hMG. Patients were planned to undergo fresh blastocyst transfer if ≥ 4 good-quality d3-embryos were available and endometrial thickness was >7 mm at oocyte retrieval. Otherwise, the d3-embryos were vitrified and transferred in an artificial FET cycle. Ongoing pregnancy rates after the first embryo transfer and cumulative ongoing pregnancy rates were calculated.
Main results and the role of chance: The overall oocyte recovery rate per follicle was approx. 50%. Forty-nine patients (25.1%) consented to donate on average 8.6 oocytes for scientific research. The maturation rate adjusted for the loss of oocytes donated for research was 49.2%. On average, 8.7 MII oocytes were available for the patient after IVM. The fertilisation rate was 66.7%. The overall OPR was 41% per cycle (80/195). No embryo was available for transfer or cryopreservation in 25 cycles (12.8%). Seventy patients had fresh transfer of 1.1 ± 0.3 blastocysts, resulting in 28 ongoing pregnancies (40.0%) and 8 clinical miscarriages (11.4%). None of the patients developed OHSS. Of the 96 patients who had a freeze-all policy, 78 did so because <4 embryos were available on day 3 after ICSI. Of these, 75 underwent at least one FET cycle. Seven patients had no FET because no embryo survived after warming. Sixty-eight patients had 1.31 ± 0.46 embryos transferred in their first FET cycle, resulting in 21 ongoing pregnancies (30.9%) and 5 clinical miscarriages (7.3%). Similar cumulative ongoing pregnancy rates were obtained after fresh + vitrified-warmed blastocyst transfers (45.7%) and after freeze all d3 strategy followed by FET (44.1%), $p = 0.85$.
Limitations, reasons for caution: This cohort analysis is based on retrospective data collection, with potential biases related to this design. Divergent treatment strategies are compared between different patient groups, depending on number of available embryos. Randomised trials are needed to demonstrate the true performance of fresh embryo transfer and FET after IVM.
Wider implications of the findings: Embryos generated after IVM have good implantation potential. The cut-off of 4 good-quality d3 embryos to perform fresh blastocyst transfer after IVM combines good clinical outcomes with low cycle cancellation rates. The observation of similar outcomes after cumulative transfer of day 3 embryos compared to blastocysts needs further scrutiny.
Trial registration number: none.

P-731 The opiate antagonist naloxone reduces the release of VEGF from primary granulosa cells of PCOS women

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Study question: How do granulosa cells from PCOS women respond to the opiate antagonist naloxone and is this affected by the presence of insulin?
Summary answer: Exposure of granulosa cells from PCOS women to naloxone induces a decrease in VEGF secretion, even in the presence of insulin.
What is known already: We recently reported for the first time that human primary granulosa cells express OPRM1, the main opiate receptor, on their surface. OPRM1 signaling appears to be functionally coupled to the VEGF signaling pathway. Blockade of OPRM1 by naloxone resulted in a decrease in granulosa cell-derived VEGF release, a phenomenon observed in both cell line (COV434) granulosa cells as well as human primary granulosa luteal cells.
Study design, size, duration: Granulosa cells from PCOS women and controls were challenged *in vitro* with the opiate antagonist naloxone, insulin or both. After 72 h of culture, the concentrations of VEGF in culture supernatant were measured by ELISA. The viable cell number in each well was assessed using a viable cell count assay (CCK-8)
Participants/materials, setting, methods: Primary granulosa cells were isolated from follicular fluid of women undergoing oocyte retrieval for IVF using a density gradient. The study included 12 women with PCOS according to Rotterdam criteria and 12 control women with male factor and/or tubal factor infertility. Granulosa cells were challenged with naloxone, recombinant insulin or both. The concentration of VEGF in culture supernatant was measured using a commercially available immunoassay. Results between groups were compared using a repeated measures ANOVA.
Main results and the role of chance: After 72 h of culture, baseline VEGF levels from PCOS granulosa were significantly higher compared to the control cohort ($p = 0.0268$). When granulosa cells were exposed to naloxone alone (2 ng/ml, 10 ng/ml), a significant reduction in the concentration of VEGF in culture supernatant was observed in both the PCOS ($p = 0.0396$, $p = 0.0003$) as well as the control groups ($p = 0.0010$, $p = 0.0098$). Incubation with insulin alone led to a significant increase in VEGF levels in PCOS granulosa cells ($p = 0.0473$), but not in granulosa cells obtained from controls ($p = 0.387$). The addition of naloxone attenuated the insulin effect, resulting in a decrease in VEGF below baseline levels ($p = 0.0087$).
Limitations, reasons for caution: The study comprised only *in vitro* experiments. Although naloxone is a highly specific OPRM1 antagonist, a low affinity for other opiate receptor subtypes cannot be excluded at the concentrations used in the experimental setup.
Wider implications of the findings: This study adds to the existing new evidence about the role of opioids in regulating granulosa cell function and demonstrates differences between PCOS and non-PCOS cells. The results hold promise for novel therapeutic options in the prevention and/or treatment of OHSS by targeting the opiate signaling system.
Trial registration number: Basic research.

P-732 A review of 300 consecutive new referrals with polycystic ovarian syndrome: presentation and management choices

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Study question: To review the demographic details, presenting symptoms, biochemical features and management in women with polycystic ovarian syndrome.
Summary answer: A significant proportion of women with amenorrhoea who met the Rotterdam PCOS definition Criteria were hypo-oestrogenic suggesting a superimposed hypothalamic component.
What is known already: Polycystic ovary syndrome (PCOS) is a common heterogeneous endocrine disorder characterized by irregular menses, hyperandrogenism, and polycystic ovaries. The prevalence of PCOS varies depending on which criteria are used to make the diagnosis, but is as high as 15%–20%. Criteria commonly used to define PCOS is that of Rotterdam consensus of 2003. Clinical manifestations include oligomenorrhoea or amenorrhea, hirsutism and frequently infertility.
Study design, size, duration: Retrospective observational study of 300 cases with PCOS referred to the reproductive endocrinology clinic in a tertiary referral hospital from 2008 to 2015.
Participants/materials, setting, methods: Electronic medical records were reviewed to screen cases of PCOS and Microsoft Excel software was used for data collection and analysis.
Main results and the role of chance: The mean (±SD) age was 30 (±6.7) years [range 14–49]. 180 (60%) women were Caucasian, 60 (20%) were Afro-Caribbean, while 60 (20%) were Asian. 226 (75.3%) were nulliparous.

A total of 213 (71%) had oligomenorrhoea, 61 (20.3%) had amenorrhoea, while 26 (8.6%) had regular cycles. 135 (45%) presented with subfertility, while 93 (31%) had hirsutism.

Mean (±SD) FSH and LH were 5.5 IU/L (±2.8) and 17.8 IU/L (±7.9), respectively, while mean (±SD) estradiol level was 253.4 pmol/L (267.1). 27/61 (44.2%) women with amenorrhoea, had low estradiol (<176 pmol/l). Three (1%) women had elevated prolactin levels. Mean (±SD) anti-Mullerian

hormone (AMH) was 44.6 pmol/L (±33.1). 39/45 (87%) had elevated AMH while 6/45 (13%) had normal levels.

Mean (±SD) testosterone level was 2.0 nmol/L (±1.5) and 22 (7.3%) patients had elevated testosterone levels. 20/43 (47% of women assessed) had elevated androstenedione levels. Lipid abnormalities was noted in 13/44 (29.5% of women assessed) and 13/28 (46.4% of women assessed) had raised HbA1C. In a total of six (2%) patients, the ovaries did not appear polycystic.

100/300 (33.3%) women received the combined contraceptive pill, 14/300 (4.6%) had progestogens, while 190/300 (63.3%) had metformin. 48/162 (16%) were referred for fertility treatment with ovulation induction.

Limitations, reasons for caution: This was an observational and retrospective study. This needs to be taken into consideration when interpreting the data.

Wider implications of the findings: The majority of cases presented with oligo amenorrhoea, approximately half of the patients presented with subfertility and there was a high prevalence of elevated adrenal androgens and abnormal lipid profile. A significant proportion of women with amenorrhoea who met the Rotterdam PCOS definition Criteria were hypo-oestrogenic suggesting a superimposed hypothalamic component.

Trial registration number: N/A.

P-733 A novel role for circulating microRNAs in the diagnosis of endometriosis and polycystic ovary syndrome

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Study question: Do blood-based circulating microRNAs differ for In Vitro Fertilization (IVF) donors compared to patients identified with endometriosis and Polycystic Ovary Syndrome (PCOS)?

Summary answer: Circulating microRNAs may provide a novel diagnostic and prognostic marker for patients with various forms of infertility diagnosis or being treated by IVF.

What is known already: MicroRNAs are endogenous small RNA molecules which regulate the expression of multiple genes. MicroRNAs have emerged as major players in the complex network of gene regulation and have been implicated in various aspects of human disease. The spectrum of microRNAs expressed in tissues differs between normal and pathologic conditions and specific patterns of microRNAs harbor diagnostic value. In addition, it has been demonstrated that microRNA modulation may have potent therapeutic effects on a plethora of human diseases. These findings have enforced the development of microRNA-based diagnostics and therapeutics in the last few years.

Study design, size, duration: The proposed study investigated differences in microRNA expression in the plasma of women undergoing controlled ovarian hyperstimulation (COH) diagnosed with PCOS [Rotterdam Criteria] and Endometriosis [Patients with laparoscopy-confirmed diagnosis] and compared them to matched Donor oocyte cycles. Samples were collected from 20 patients from each group at the baseline of their cycle (Days 3–5) to initially identify candidate microRNAs for the different etiologies of patients.

Participants/materials, setting, methods: Total RNA was extracted from plasma samples collected on Day 3 to 5 of the cycle. The quality and integrity of the RNA samples was evaluated using the Agilent Bioanalyzer 2100. One hundred nanograms of each RNA sample was used to perform the microRNA high throughput analysis by using the Nanostring nCounter Platform enabling us to evaluate the expression levels of 800 specific microRNAs. A select number of candidate microRNAs were then validated using qPCR.

Main results and the role of chance: The analysis compared Control vs. Endometriosis, Control vs. PCOS and Endometriosis vs. PCOS. Probes were recognized as expressed if there were >25 counts in >25% of samples. We found 353 probes that passed these criteria and were subsequently used for further analyses. *T*-tests were performed to assess significance of differences between expressions. Control vs. Endometriosis and Control vs. PCOS identified 29 and 20 significantly [range $P = 0.003–0.045$] differentially expressed microRNAs, respectively. Endometriosis vs. PCO identified 21 significantly [range $P = 0.001–0.045$] different expressed microRNAs. Further confirmatory analysis initially involved selecting 2 upregulated and 2 downregulated microRNAs from each comparison. All these comparisons confirmed significantly different expression of specific microRNAs, including miR-1249, which was up-regulated in both endometriosis patients (2.2-fold) and PCOS patients (2.7-fold) relative to control samples. Furthermore, miR-555 was found to be increased 2-fold in endometriosis relative to PCOS patients. The identification of differentially expressed microRNAs by the nCounter technology and their further validation by qPCR provides encouragement that these are indeed candidate microRNAs, however further analysis of their particular pathways is needed to rule out misidentification of individual microRNA candidates.

Limitations, reasons for caution: The microRNAs identified in this initial study need to be validated in a larger subset of patients and in a blinded analysis to confidently accept their authenticity. A further limitation is that these results are correlated to our current diagnostic assumptions of both PCOS and endometriosis.

Wider implications of the findings: Improving our ability to non-surgically predict and diagnose various infertility pathologies will significantly impact the practice of IVF. The data can also contribute to improve our understanding of the complex signaling pathways involved in PCOS and endometriosis and assist in developing novel alternatives for treatment.

Trial registration number: Not Applicable.

P-734 The advantage of an immediate frozen embryo transfer following a freeze-all protocol: a retrospective analysis from 2 centres

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Study question: Does the timing of the first frozen embryo transfer (FET) after gonadotropin-releasing hormone (GnRH) agonist triggering with the elective cryopreservation of all embryos affect pregnancy outcome?

Summary answer: FETs performed immediately after a freeze-all cycle were associated with higher pregnancy rates.

What is known already: As the interest and use of the freeze-all strategy expand in the field of reproductive medicine, the optimal timing to perform the subsequent FET has become increasingly important. Thus far, all clinical trials evaluating the efficacy of the segmentation strategy opted to electively defer the first FET for at least one menstrual cycle. However, this merely empirical approach may cause unnecessary distress to infertile patients eager to conceive as soon as possible.

Study design, size, duration: This retrospective cohort study included the first FET cycle of all women who underwent a freeze-all protocol between October 2010 and October 2015 in two reproductive medicine centres.

Participants/materials, setting, methods: A total of 344 FET cycles (performed in 335 patients) were included in the analysis. Following the freeze-all cycle, the preparation of the endometrium consisted of the sequential administration of estradiol valerate and micronized vaginal progesterone. The start of the FET was classified as either immediate (≤ 14 days after oocyte retrieval) or delayed (>14 days after oocyte retrieval). Clinical pregnancy rate (CPR) was the main outcome of our study.

Main results and the role of chance: No significant differences were found between the immediate and delayed FET groups regarding female age, number of oocytes retrieved, indication for the freeze-all protocol (specifically, high-risk of ovarian hyperstimulation syndrome, late-follicular progesterone >1.5 ng/mL, late-follicular endometrium <7 mm or other reasons), and the number, developmental stage and quality of the embryos transferred at FET. The CPR was significantly higher in the immediate FET group (54.0% after immediate FET vs. 39.7% after deferred FET, $p = 0.009$), even after accounting for potential confounding with the use of mixed-effects multilevel multivariable logistic regression analysis (adjusted odds ratio 0.54, 95% confidence interval 0.34–0.85, $p < 0.001$).

Limitations, reasons for caution: The results are limited by the retrospective design and the potential for unmeasured confounding. Furthermore, this study only evaluated the effect of FET timing on CPR in artificially-supplemented cycles and, thus, the results should not be extrapolated to live birth rates or natural-cycle FETs.

Wider implications of the findings: This study offers a simple but potentially relevant measure to increase the patient-satisfaction and adherence in couples who seek to become pregnant both safely and as soon as possible.
Trial registration number:/

P-735 Comparing the inter-cycle variation of serum anti-Mullerian hormone and antral follicle count measurements in predicting ovarian response before IVF

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Study question: Does serum anti-Mullerian hormone (AMH) have less inter-cycle variation than antral follicle count (AFC) when used as an ovarian response predictor?
Summary answer: Inter-cycle variations were significantly less with serum AMH than AFC measurement, although both have no significant difference in their predictive performance on poor ovarian response.
What is known already: Both AMH and AFC measurements are recognized as the best markers for predicting ovarian response before IVF treatment. There was one report in the European population indicating that AMH (measured using the older ELISA) had less inter-cycle variation than AFC. However, there is no report on this in the Asian population nor using the newer generation ELISA.
Study design, size, duration: This observational study was a secondary analysis using AMH and AFC data from control subjects of another prospective clinical trial on the effect of DHEA pre-treatment. Only control subjects ($n = 45$) in the placebo arm were included in this secondary analysis. AMH and AFC measurements over four consecutive menstrual cycles before they underwent standard IVF treatment using the GnRH antagonist protocol were analysed.
Participants/materials, setting, methods: The subjects underwent IVF treatment in a university-affiliated hospital in Hong Kong. AFC was measured in the early follicular phase (between days 2–5) by transvaginal ultrasonography in four consecutive cycles, and serum was obtained on the same occasions for AMH measurement using the Gen II AMH ELISA (Beckman-Coulter). The intra-class correlation coefficients (ICC) for AFC and AMH across the four study cycles, as well as their predictive performance on poor ovarian response, were compared.
Main results and the role of chance: Subjects were aged between 30 and 40 years. No significant difference was observed in AFC ($p = 0.114$) and AMH ($p = 0.375$) across the four study cycles (Friedman's test). The single-measures ICC of AFC and AMH were 0.597 (95% CI 0.459–0.726) and 0.850 (95% CI 0.778–0.906) respectively. The average-measures ICC of AFC were 0.856 (95% CI 0.773–0.914) and 0.958 (95% CI 0.933–0.975), respectively. Hence, both single-measures and average-measures ICC were significantly higher with AMH than with AFC. The areas under the ROC curve of the four AFC measurements in predicting poor ovarian response (defined as three or less oocytes retrieved) in the subsequent IVF cycle ranged from 0.693 (95% CI 0.536–0.844) to 0.818 (95% CI 0.672–0.918) with no significant difference ($p > 0.05$) between the four cycles, whereas those of the four AMH measurement ranged from 0.785 (95% CI 0.636–0.895) to 0.821 (95% CI 0.676–0.920) with no significant difference ($p > 0.05$) between the four cycles.
Limitations, reasons for caution: This was a secondary analysis on data obtained from another clinical trial; no data on intra-cycle variation of AFC and AMH was available.
Wider implications of the findings: Our study in the Chinese population using the newer generation AMH ELISA concurred with the findings from a previous European study. Although both AFC and AMH are good predictors of ovarian response, AMH is less subjected to inter-cycle variation than AFC, hence allowing pre-IVF assessment at more flexible timing.
Trial registration number: NCT01915186 (www.clincialtrials.org).

P-736 Intracellular oxidative stress in granulose cells impact on ART success in reduced ovarian reserve

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Study question: To assess the relationship between intracellular reactive oxygen species (ROS) levels in granulose cells (GCs) and assisted reproductive techniques (ART) outcome parameters in reduced ovarian reserve-ART patients.
Summary answer: High levels of intracellular oxidative stress (OS) in GCs have a negative impact on ovarian reserve and ART outcomes.
What is known already: ROS play physiological roles during folliculogenesis and oocyte maturation but excessive ROS production may create an unsuitable environment for reproduction and be related to pathological conditions. ART outcome is adversely affected if an imbalance exists between ROS and antioxidants in the oocyte microenvironment. In PCOS- ART cases, OS mediated alterations in GCs negatively influences ART success. So, OS might be responsible for reduced ovarian reserve in young women who were admitted to ART treatment. To our knowledge, no study examined the correlation between intracellular ROS levels in GCs and ART outcome parameters in low responder patient.
Study design, size, duration: This prospective study compared ART outcome parameters (oocyte quality, fertilization rate and embryo quality) with intracellular ROS in GCs percentage of 15 patients 35 years, expected low responders (defined as having AMH ≤ 1 ng/L) (A Group) and 15 patient <35 years, expected normal responders (AMH >1 ng/L) (B Group) undergoing ICSI-ET cycles during the period from March to October 2015.
Participants/materials, setting, methods: Exclusion criteria: PCOs, endometriosis, metabolic and endocrinology diseases, severe Oligoasthenoteratozoospermia. GCs samples were isolated by centrifugation from follicular fluid that was obtained on the day of oocyte retrieval. The intracellular ROS levels were assessed by H2DCF-DA (2',7'-dichlorofluorescein diacetate) fluorescent probe. Oocyte quality, fertilization rate and embryo quality outcome were assessed. The data were analyzed using the unpaired Student's *t*-test and considered significant if p-value ≤ 0.05.
Main results and the role of chance: Statistically significant difference between the two groups in intracellular ROS levels in GCs have been observed. In particular, ROS percentage was significantly higher in young women with reduced ovarian reserve compared normal responders ($p \leq 0.05$) (34 ± 5.5 vs. 19 ± 7.8). The B group showed a better oocyte quality associated with a higher percentage of fertilization and embryo development compared to A group ($p \leq 0.05$). In detail, the average percentage of MII oocytes recovered was 75% ± 6.7 and 86% ± 8.4 in poor responder and normal responder patients respectively ($p \leq 0.05$). Among A and B Group, a significant statistical difference was recorded in the average percentage of fertilization (72% ± 6.0 vs. 92% ± 3.0). The average percentage of good quality (grade 1–2) embryos on day 3 in patients with reduced and normal ovarian response was 71% ± 4.2 and 87% ± 5.5 respectively ($p \leq 0.05$). High levels of ROS in GCs were significantly negatively correlated with ART outcome parameters.
Limitations, reasons for caution: Small number of participants. Lifestyle factors (i.e., smoking, diet) are not considered.
Wider implications of the findings: This research showed that increased oxidative stress in GCs is a possible explanation for low ovarian response in young women. The demonstrated excessive ROS production has negative implication in ART fertility outcome. These results could lead to the development of specific antioxidant therapies for women with poor prognosis undergoing ART.
Trial registration number: No. 29 (19/01/2015).

P-737 Reproductive outcomes in patients with polycystic ovaries: does phenotype matter?

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Study question: Do live birth rates vary in women with different Rotterdam PCOS phenotypes who undergo IVF/ICSI treatment with fresh embryo transfer?

Summary answer: Patients with Rotterdam PCOS are a heterogeneous group with divergent outcomes after IVF/ICSI. Hyperandrogenemia seems to be associated with a significantly poorer outcome.
What is known already: There is increasing evidence from reproductive endocrinology research that PCOS patients with Rotterdam criteria constitute a heterogeneous population with distinct risk profiles of metabolic, reproductive and obstetric complications. However, the impact of the PCO/PCOS phenotype on reproductive outcomes after controlled ovarian stimulation on a fresh embryo transfer is currently unknown.
Study design, size, duration: This was a retrospective cohort study using clinical and laboratory data from 330 PCO/PCOS patients with AMH levels above the cut-off for predicted hyperresponse, i.e., > 20 oocytes, undergoing their first IVF/ICSI treatment cycle using a GnRH antagonist protocol in a tertiary reproductive centre. Data were collected between January 2010 and December 2014. Patients with unspecified PCOS phenotype and those requiring either PGD or testicular biopsy were excluded.
Participants/materials, setting, methods: Patients were divided into four phenotype groups: women with isolated increased serum AMH levels (n = 102) without cycle irregularities, mild PCOS presenting with isolated increased serum AMH levels and either oligo/amenorrhoea (PCOS-OA, n = 129) or hyperandrogenemia (PCOS-HA, n = 35), and full-blown severe PCOS with all of three Rotterdam criteria (n = 64). The primary endpoint of the study was live birth rate (LBR). Secondary endpoints included consumption of gonadotropins, duration of stimulation and incidence of OHSS.
Main results and the role of chance: The distinct groups had similar baseline characteristics except for the body-mass index (BMI). The mean BMI (kg/m^2 ± standard deviation) was significantly higher in severe PCOS patients (29.1 ± 5.6; $p < 0.001$) compared to women with isolated increased AMH (24.2 ± 5.1), PCOS-OA (23.6 ± 4.6) and PCOS-HA (27.0 ± 5.6).

The LBR in women with isolated increased AMH, PCOS-HA, PCOS-OA and severe PCOS were 35.3%, 14.3%, 33.3% and 12.5%, respectively (Pearson χ^2, $p < 0.001$). Pairwise comparisons revealed that LBR were significantly lower in women with the severe PCOS phenotype when compared to those with isolated increased AMH ($p = 0.002$) and PCOS-OA ($p = 0.003$). The χ^2 test for trend was significant ($p = 0.02$).

Multivariate logistic regression analysis accounting for the following confounding factors was also performed: BMI, age, fertilisation rate, day of embryo transfer in the fresh cycle and insemination method (IVF, ICSI or IVF/ICSI). In this analysis, PCOS phenotype remained an independent predictive factor ($p < 0.02$) for LBR.

The total consumption of gonadotropins was significantly higher in patients with severe PCOS whereas the duration of stimulation, number of retrieved oocytes and fertilisation rate did not differ significantly among the groups. Seven patients (2.1%) developed OHSS requiring hospital admission: 2 women with isolated elevated AMH, 3 from the PCOS-OA and 2 from the severe PCOS group.
Limitations, reasons for caution: These results should not be extrapolated to PCO/PCOS patients with normal AMH-levels. Furthermore, only biochemical hyperandrogenemia (and not clinical hyperandrogenism) was considered in this study. Another limitation is the retrospective design and the relatively small sample size of the PCOS-HA subgroup. Anyhow, these results warrant confirmation in future studies.
Wider implications of the findings: The lower LBR in patients with severe PCOS, compared to other PCOS phenotypes, is potentially related to the impact of BMI or metabolic profile on oocyte and endometrium quality. These preliminary findings may one day imply the need for a tailored approach when treating PCOS patients with hyperandrogenemia.
Trial registration number:/

P-738 How to implement in real-life settings the results of meta analysis? Example of dydrogesterone as luteal phase support after fresh embryo transfer in IVF procedure

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Study question: The primary objective was to compare in an unselected population the live birth rate after fresh embryo transfer either with oral dydrogesterone or vaginal progesterone for luteal phase support (LPS).
Summary answer: In an unselected population dydrogesterone and vaginal progesterone for LPS after fresh embryo transfer showed similar live birth rates.
What is known already: Randomized controlled trials and meta analysis demonstrate that LPS after ovarian hyperstimulation, oocyte pick up and embryo transfer is mandatory for the best success in IVF cycle and that different routes of progesterone show the same efficacy. However these studies are performed in selected populations and the worlwide application of these findings remains weak, especially for oral progesterone. The most used route remains the vaginal one, despite the discomfort for the women. But changing the routine in a clinical practice is challenging, specifically in ART procedures, which success depends on a large range of factors.
Study design, size, duration: This is a prospective cohort study including all the women undergoing a fresh embryo transfer after autologous IVF procedure, in one public IVF center for 16 months, from December, 2011 to march, 2013. To get real-life data, the size of the cohort was estimated to be at least 500 patients, which represents approximately one year practice in an average setting in our country.
Participants/materials, setting, methods: All the 559 consecutive fresh embryo transfers during the study period were included, no women were excluded, whatever demographic data were. The progesterone supplementation was introduced the day of oocyte pick up. As both treatments were expected to be as effective, women were treated with micronized vaginal progesterone 200 mg twice a day when starting on even day (N = 291) or oral dydrogesterone 10 mg twice a day when starting on uneven day (N = 268).
Main results and the role of chance: The two groups were comparable regarding the demographic data (age, BMI, duration of infertility), stimulation protocols (IVF/ICSI, agonist/antagonist, total dose of FSH) and stimulation outcomes (number of oocytes retrieved, metaphase2 oocytes, total embryos and embryos replaced). However, there was a significative difference in the rank of IVF procedure 1.73 ± 0.96 vs. 1.54 ± 0.80 (p = 0.004) and number of embryos cryopreserved 3.0 ± 2.2 vs. 3.7 ± 3.0 (p = 0.006), in vaginal progesterone and dydrogesterone groups respectively. There were no significant differences in clinical pregnancy, implantation and birth rates between the vaginal and oral groups, 31.3% vs. 27.2% (p = 0.29), 18.6% vs. 17.1% (p = 0.49) and 24.1% vs. 22.4% (p = 0.64) respectively. The miscarriage rate was also similar in both groups 23.1% vs. 17.8% respectively. A subgroup analysis was performed in good prognosis patients (rank 1 or 2 and < 35 years old; N = 274): all the demographic and stimulation data were comparable in both groups. The clinical pregnancy rate and birth rate were similar in vaginal and oral groups, 35.9% vs. 31.7% (p = 0.48) and 26.6% and 30% (p = 0.55) respectively. But miscarriage rate was significantly higher in the vaginal progesterone group 26.1% vs. 5.3% in the oral group (p = 0.01).
Limitations, reasons for caution: This is a real-life setting study with practical randomization, the limitation to the conclusion is bias for studying non comparable groups regarding the demographic data or the protocols used, which is currently the case. This can be addressed with subgroups analysis with sufficient number of patients in different groups.
Wider implications of the findings: Although this is not a registered indication, dydrogesterone was as effective and safe as vaginal progesterone, not only for good prognosis patients. The benefits of oral over vaginal route are observance and tolerance, especially in the group of younger patients, less dedicated to the effort to continued treatment for LPS.
Trial registration number: Not required.

P-739 Defining critical progesterone elevation to estimate effect on pregnancy and live birth rates: influence of selected threshold on exposure frequency, effect size and clinical impact.

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Study question: To evaluate the influence of defining threshold for progesterone elevation in final clinical impact, in terms of lost clinical pregnancies and live birth rate reduction.

Summary answer: Defining thresholds of progesterone elevation determine an inverse association between elevation frequency and effect size, so final impact on IVF outcomes is discrete.
What is known already: Early progesterone elevation (EPE) has been described as unfavorable side-effect of ovarian stimulation that can disregulate endometrial receptivity and embryo implantation rate in IVF. Controversy about the critical level of progesterone serum level on day of HCG that could be detrimental for IVF outcomes is still remaining. Lack of consensus about this issue has generated an important heterogeneity in defining thresholds of EPE, which has obvious consequences on described frequencies and sizes of effect.
Study design, size, duration: Retrospective cohort study of an opportunity sample of 3729 IVF cycles with fresh embryo transfer, carried out over an 8-year period. Cycles with data about final progesterone serum concentration and pregnancy outcome were included. Attrition rate due to missing data that impeded inclusion of observations in multivariate analysis was quantified.
Participants/materials, setting, methods: We analyzed 3729 IVF cycles from Clínica Tambre, an specialized reproductive centre in Madrid (Spain). After identifying potential progesterone thresholds able to decrease IVF outcomes by interval analysis, we performed univariate estimation of effect size for each one. Multivariate analysis identified independent associations with outcome variables. Crude and adjusted risk differences were used to estimate NNH and final impact of each threshold of elevated progesterone on pregnancy and live birth rate loss.
Main results and the role of chance: Serum progesterone level on HCG day was significantly and proportionally associated with clinical pregnancy and live birth rate within a concentration range, including levels from 1.26 ng/mL to 1.81 ng/mL and from 1.26 ng/mL to 1.65 ng/mL, respectively. Relative risk (RR) for clinical pregnancy declined progressively from lower to upper critical thresholds considered to define early progesterone exposure: estimated RR for clinical pregnancy varied from 0.73 (CI 95%: 0.61–0.88, $p < 0.001$) for progesterone levels >1.26 ng/mL (177 cycles) to 0.52 (CI 95%: 0.35–0.77, $p < 0.001$) for expositions to progesterone >1.65 ng/mL (112 cycles).

Similarly, effect size for live birth decreased from estimated RR of 0.75 (CI 95%: 0.6–0.95, $p = 0.004$) for progesterone >1.26 ng/mL (357 cycles) to 0.48 (CI 95%: 0.27–0.78) for progesterone exposure upper to 1.65 ng/mL. The harmful effects of progesterone elevation remained after adjusting by other independent covariates.

The inverse relation between selected threshold and frequency of progesterone elevation determined a linear and parallel increase of progesterone frequency and number of cycles needed to harm (NNH) for clinical pregnancy (10.6 to 6.5) and for live birth (NNH: 15.6 to 7.2). Anyway, final number of estimated lost clinical pregnancies and live births were low and relatively unchanging.
Limitations, reasons for caution: The retrospective approach of our study could be considered as a limitation. Additionally, multivariate analysis has been used to reduce risk of bias effects, but they can not be absolutely excluded.
Wider implications of the findings: Accordingly with previous studies, endometrial progesterone exposure appears detrimental for IVF effectivity but only above extreme values. Although highest levels of exposure are related to relevant decreases of pregnancy and live birth rates, these exposures affects only a small proportion of cycles, so final impact could be considered low.
Trial registration number: No applicable.

P-740 The most effective starting dose of gonadotropins in the first IVF cycle in patients under 35 years of age – GnRH agonist versus antagonist protocol.

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Study question: What is the most effective starting dose of gonadotropins in the first IVF cycle in patients under 35 years of age – GnRH agonist vs. GnRH antagonist protocol.
Summary answer: The most effective starting dose of gonadotropin for GnRH agonist protocols is 150 IU in comparison to <150 IU per day in antagonist protocols.
What is known already: A significant number of randomized controlled trials were identified the question of the most effective FSH starting dose in expected normal responders. The most of results suggested a maximum effective dose of recombinant gonadotropin for the first IVF cycle for 300 IU/day, with a starting dose of 150 IU per day.
Study design, size, duration: Retrospective observational study on 3116 fresh embryo transfers, during the decade, in patients in the first IVF cycle. Analysis covers clinical data, embryological data and outcomes of fertility treatments.
Participants/materials, setting, methods: 3116 patients in GnRH agonist or GnRH antagonist protocol with mild male or tubal factor of infertility were included to the study. Only patients with good ovarian reserve were included to the study. The number of fertilized eggs was limited to 8 and single embryo transfer was performed. All cycles were divided according to the starting dose of gonadotropins <150 IU, 150–224 IU, 225–299 IU, >300 IU and type of protocol – GnRH agonist and antagonist.
Main results and the role of chance: The highest clinical pregnancy rate per embryo transfer 60.2% was observed at starting dose of gonadotropins 150–224 IU in long GnRH agonist protocol. In GnRH antagonist protocol the highest clinical pregnancy rate 59.3% were observed at starting dose <150 IU. The lowest clinical pregnancy rate per embryo transfer 37.7% was observed at starting dose of gonadotropins <150 IU in long GnRH agonist protocol. In GnRH antagonist protocol the lowest clinical pregnancy rate 47.7% were observed at starting dose >300 IU. The highest mean consumption of gonadotropins was observed in long GnRH agonist protocol (2946.21 IU) with mean time of stimulation 10.2 days (starting dose >300 IU). The lowest consumption of gonadotropins was observed in GnRH antagonist protocol (1217.21 IU) with mean time of stimulation 9.1 days (starting dose 150–224 IU).
Limitations, reasons for caution: This is only a data presenting clinical pregnancy outcome. The results will be completed when all available live-birth data can be analyzed, as well as miscarriages and preterm deliveries rate.
Wider implications of the findings: The results can be helpful to presents the best compromise between safety and efficacy of treatment for the younger IVF patient with a good prognosis depending on the type of controlled ovarian hyperstimulation protocol.
Trial registration number: N/A.

P-741 Anti-Müllerian hormone (AMH) serum levels enable preoperative estimation of the number of cumulus-oocyte complexes (COC) in female-to-male transgender persons (trans men)

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Study question: Can we correlate the number of COC in ovaries of trans men at the time of ovarian tissue cryopreservation to clinical or biochemical markers?
Summary answer: AMH correlates significantly with the number of recovered COC, enabling to determine an average estimated increase of COC by 7.48 per unit AMH.
What is known already: Ovarian cortex cryopreservation is a standard technique for fertility preservation in trans men. During tissue manipulation for ovarian cortex freezing, COC are possibly released from antral follicles. It has been shown, in an oncologic patient cohort, that these immature oocytes can be recovered from the antral follicles and subsequently *in vitro* matured and cryopreserved (Huang et al., 2008; Fasano et al., 2011; Segers et al., 2015). The presence of these immature oocytes nor possible clinical or biochemical markers are thus far confirmed during the processing of ovaries originating from trans men.
Study design, size, duration: From June 2013 to December 2014, 30 trans men (mean age 24 + 6 years, mean duration of testosterone treatment

59.0 + 23.8 weeks) were included after informed consent. Patient characteristics and hormone serum levels were collected. Following ovarian cortex processing for cryopreservation, the manipulation dishes were examined under a stereomicroscope for COC. To validate our findings, from February 2015 to January 2016 we included 9 trans men (mean age 22 + 3 years, mean duration of testosterone treatment 60.4 + 40.3 weeks).

Participants/materials, setting, methods: The hormone levels were determined using a E170 Modular® (Roche Diagnostics, Mannheim, Germany), except for AMH serum levels (A73818, Elisa Immunotech, Beckman Coulter, Woerden, Netherlands). For validation of our finding, AMH serum levels of 9 patients were determined using the AMH Roche E170 (Roche Diagnostics, Mannheim, Germany). Statistical analysis for correlation (Spearman's rank-order correlation test) and linear regression analysis was performed with IBM SPSS Statistics 23.

Main results and the role of chance: In total, 1087 COC were collected from the first patient cohort ($N = 30$). A mean of 36.23 + 35.81 COC per patient were retrieved. AMH serum levels correlated strongly with the amount of COC (Rs 0.787, $p < 0.001$). The number of COC did not correlate with other hormone serum levels nor with duration of testosterone treatment. Next, linear regression analysis showed an equation $y = 0.847 + 7.48x$ (R^2 0.714) with a non-significant intercept (b0 = 0.847; $p = 0.875$; 95% CI −10.44–12.19) but a significant increase of the estimated mean number of COC with 7.48 per unit AMH (µg/L) ($p < 0.001$, 95% CI 5.73–9.23). Validation of the average estimated increase of 7.48 COC (95% CI 5.73–9.23) per unit AMH (µg/L) was confirmed on the second patient cohort. This equation could correctly predict the number of COC in 44.44% (4/9) patients but underestimated the COC yield in 44.44% (4/9) patients. The underestimation ranged from 36.95 estimated vs. 60 retrieved COC to 7.55 estimated vs. 32 retrieved COC. In case of the overestimation, only 22 COC were collected where 49.14 COC were calculated.

Limitations, reasons for caution: The COC yield in this study did not show a correlation with testosterone serum levels nor with the duration of the testosterone therapy. Nevertheless, the influence of hormonal treatment in transgender persons on the ovary is currently not fully understood.

Wider implications of the findings: An estimation of the COC yield in function of AMH could be of importance during the counseling of patients for fertility preservation options. Our results clearly indicate that the average estimated increase is assay specific and should therefore be calculated before use in daily practice based on the available assay.

Trial registration number: UZ Ghent reference: 2012/780 – Belgian registration number: B670201215468.

P-742 Start stop protocol: A preliminary study of a new controlled ovarian stimulation method for Bologna poor responders

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Study question: Is there any clinical importance for follicular improvement of 1 week later control of Bologna poor responder patients unresponsive to initial controlled ovarian stimulation (COS) in IVF/ICSI cycle**.**

Summary answer: Start Stop Protocol is a new and effective alternative method for Bologna poor responders unresponsive to initial 1 week COS in IVF/ICSI cycle.

What is known already: Although many protocols with different doses and types of gonadotropins have been proposed in the literature, to date, there is insufficient evidence to identify the use of any particular intervention to improve treatment outcomes in poor responders.

Study design, size, duration: This retrospective uncontrolled pilot study was conducted in IVF units of two university hospitals. A total of 375 patients fulfilling the Bologna poor responder criteria underwent COS (recFSH plus hphMG) and unresponsive to initial gonadotropins were included in the study between January 2015 and January 2016.

Participants/materials, setting, methods: Forty three of the 375 patients (11.5%) unresponsive to about 1 week initial COS in IVF/ICSI cycle were included in the study. After stopping all gonadotropin medications, the patient was requested for ulrasonography and serum estradiol control. If follicular improvement and/or increase in estradiol level were detected, patient underwent COS using antagonist protocol. Once follicle diameter >17 mm, hCG was administered and the oocyte collected 36th after hCG.

Main results and the role of chance: Baseline demographic and clinical data: Mean age 36.3 ± 5.02 years, body mass index 26.02 ± 4.68 kg/m^2, infertility period 6.47 ± 4.81 years, basal FSH 17.7 ± 12.6 mIU/ml, basal estradiol 32.9 ± 40.6 pg/mL, median number of day 2 antral follicul count 2 (0–6), hCG day follicle >17 mm 1 (0–4), stimulation time until COS stop 8 (4–14) days, stimulation time after COS restart 5 (0–11) days, number of MII oocyte 1 (0–4), number of 2PN embryo 1 (0–4). Nineteen of 43 patients (44.2%) achieved MII oocyte yield and seventeen of 43 patients (39.5%) achieved embryo transfer. Clinical pregnancy rate was 29.4%.

Limitations, reasons for caution: Retrospective design, small sample size and having no control group are major limitations of this study. However, this is a preliminary report of a new treatment strategy for Bologna poor responder patients.

Wider implications of the findings: Further prospective controlled studies are needed to show effectiveness of Start Stop Protocol for Bologna poor responder patients unresponsive to initial COS in IVF/ICSI cycle**.**

Trial registration number: N/A.

P-743 Long-term health of women with FSH resistant ovaries

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Study question: Do inactivating FSH receptor mutation and hypoestrogenism have long-term effects on health and morbidity, and can these be overcome by an adequate hormone therapy (HT)?

Summary answer: All women with FSH resistant ovaries (FSHRO) in this cohort had been on HT and had no major health problems related to the mutation.

What is known already: Autosomal, recessively inherited FSH receptor mutation underlies FSHRO syndrome, a genetic form of primary ovarian insufficiency (POI). FSHRO patients have low endogenous estrogen levels, which has potential effects on health and morbidity. In addition, FSH resistance arrests follicle development at antral stage leading to infertility. The inactivating FSH receptor mutation was discovered 20 years ago, and due to the relatively short follow-up period so far, its long-term effects on health and morbidity, and the effect of HT have not been thoroughly investigated.

Study design, size, duration: A cohort study of 26 women with a confirmed inactivating FSH receptor mutation (A189V). Twenty-two of them (85%) filled and returned a questionnaire.

Participants/materials, setting, methods: Twenty-two women aged 28–70 years participated in this study. The questionnaire included detailed questions regarding the use of HT, infertility treatments and their success, bone density measurements and incidence of osteoporosis, and cardiovascular symptoms and disease. In addition, the patients were asked to report any malignancies, operations, and neurological, psychological, respiratory, gastro-intestinal, cutaneous, or allergy symptoms.

Main results and the role of chance: All women had used and currently 46% were using HT. 14% of the women were also using local vaginal estrogen therapy. The median starting age for the use of HT was 18 years and the median time of use was 20 years. 14 women (63%) had undergone infertility treatments *via* ovum donation, and 10 women had become pregnant, four of them twice. The outcomes were one miscarriage, 11 singleton pregnancies and two twin pregnancies. Overall 15 children have been born. The pregnancies were largely uneventful. A bone density measurement (DXA) had been performed on 16 (73%) of FSHRO patients, and only one person was diagnosed with osteoporosis. Seven women were currently under hypertensive medication, but no coronary heart disease was reported. Four women were using asthma medication, and four were on proton-pump inhibitor medication. No malignancies were reported, and only one woman had been diagnosed with a cervical dysplasia.

Limitations, reasons for caution: The information on health and morbidity was self-reported.

Wider implications of the findings: Early-onset HT seems to be effective in preventing major long-term effects on health and morbidity in a genetic form

of POI. Ovum donation is an effective treatment of infertility in FSH resistant ovary syndrome.
Trial registration number: The Hospital District of Helsinki and Uusimaa Ethics Committee for gynaecology and obstetrics, pediatrics and psychiatry 333/13/03/03/2013.

P-744 Different pregnancy rate with the same results of AMH using 5 different AMH kits including new automated assays

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Study question: The aim of that retrospective study was to compare embryological and clinical outcome with respect to five different AMH kits.
Summary answer: This study demonstrated that we could expect totally different pregnancy rate with the same results AMH using different AMH kits.
What is known already: Ovarian reserve is an important parameter for assessment of the chances for successful infertility treatment and AMH is important, widely used parameter for its determination. Based on AMH results decisions regarding gonadotropins dosage and pregnancy prediction in IVF for each individual patients are made. Few kits are available on the market for determination of AMH concentration. Some studies presented different AMH values with different kits – but the vast majority was limited to a small number of patients. This is why we decided to compare five kits regarding the pregnancy rate with a large cohort.
Study design, size, duration: The study population consisted of 3693 patients treated by ICSI in our IVF Center between 2007 and 2015. For retrospective analysis, we excluded women that participated in the oocyte donation, enterprise program, e.g., those with frozen–thawed embryo transfers. We divided women according to age and AMH categories.
Participants/materials, setting, methods: In this study, we compared AMH serum concentrations using five tests. All women underwent controlled ovarian stimulation. Embryo transfer was performed on blastocyst stage on day 5. Number of embryos transferred was determined by their availability and quality, and the guidelines of the institution and ASRM. Each patient was tested only once using one kit.
Main results and the role of chance: Immunotech I Gen. RUO (n = 1451), Beckman Coulter II Gen. RUO (n = 652), Beckman Coulter II Gen. IVD (n = 214), Ansh Labs I Gen. IVD (n = 897), Elecsys Roche (n = 479) assays were investigated.

Assays differed significantly in the meaning of pregnancy rate achieved. In women <35 years with AMH concentrations <0.6 ng/ml and above 1.4, and in those >39 years with AMH concentrations between 0.6 and 1.4 and above 1.4, the clinical pregnancy rate differed significantly between AMH kits. In both aforementioned subgroups, the higher rate occurred with the Beckman Coulter II Gen and Ansh Labs. IVD, beside group >39 and >1.4 where the Elecsys Roche group exhibit highest value. The lowest pregnancy rate was seen with the IOT.

When we divided women according to their age category, the highest pregnancy rate was observed in women <35 years. In that case, the higher clinical pregnancy rate was found with the Beckman Coulter II Gen. IVD, while the lowest was found with the IOT.

AMH concentrations also differed significantly between different kits. In groups of women <35 and those in age 35–37, the highest value was found for IOT, and the lowest for Beckman IVD.
Limitations, reasons for caution: Limitation was retrospective nature of study that could be a bias for the achieved results in terms of pregnancy rate. On the other hand our pregnancy rate was quite stable during investigated period.
Wider implications of the findings: In summary, these results could have potential importance for clinical decision making as AMH levels are frequently used to determine gonadotropins dosing in IVF.
Trial registration number: Not applicable.

P-745 Serum Prolactin and Cortisol levels for luteal phase support (LPS) during IVF: subcutaneous vs. intramuscular progesterone

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Study question: Investigate whether LPS with IM and SC progesterone determine changes in the stress hormones (Cortisol and Prolactin), that are known to influence IVF outcomes.
Summary answer: The values of Prolactin and Cortisol were statistically significantly higher in group treated with IM route compared to the group treated with SC route.
What is known already: The stress role in IVF has long been a topic of interest. Questions include whether the treatment is stressful, whether stress or anxiety has an impact on success of fertility treatment and whether interventions to decrease stress are useful.

LPS in IVF is necessary and recommended, because it has been shown that in stimulated cycle there is an abnormal luteal phase, leading to poor endometrial development and an asynchrony of endometrial receptivity.

Recently, it has become available a water-soluble injectable progesterone, that makes possible the subcutaneous (SC) injection for which patients are already familiar for daily treatment with gonadotropins.
Study design, size, duration: This is a prospective study that include 70 women undergoing their first IVF treatment for primary infertility from February to December 2015; they were randomized in two groups with different way of administration of LPS: A group (35 patients) receive 33 mg/day of IM in oil-progesterone starting from pick-up day and than 50 mg/day from embryo transfer (ET) day; B group (35 patients) receive 25 mg of SC water soluble-progesterone from pick-up day.
Participants/materials, setting, methods: Were excluded: acute illness, chronic disorders, PCOs, Endometriosis, organic dysfunction. Patients underwent ovarian stimulation with a long protocol. Blood samples were obtained from all subjects at day + 7 from oocyte retrieval. CORT and PRL serum levels were obtained by Immulite from DPC/Siemens (Princeton, NJ). The outcome was the comparison between patients treated with IM and with SC progesterone. For statistical analysis, t-test, χ^2, log rank analysis, and analysis of variance were used as appropriate.
Main results and the role of chance: All patients completed IVF stimulation, egg retrieval, ET, and resultant pregnancy tests. Group A and B were similar concerning baseline characteristics and stimulation data. All patients have a regular lifestyle during LPS, not experiments bleeding or spotting and any health problem. The values of PRL and CORT were statistically significantly higher in group treated with IM route compared to the group treated with SC route (respectively 27.5 ± 3.3 vs. 21.8 ± 3.3, $p < 0.001$ and 345.5 ± 29.9 vs. 310.5 ± 28.7, $p < 0.001$).
Limitations, reasons for caution: A limit of our study is the small number of patients enrolled. By the way, we want to point out that this experience represents a pilot study, on the basis of which we hope to design a more important study to validate our results.
Wider implications of the findings: Our results show for the first time that treatment with SC progesterone is associated with lower Cortisol and Prolactin levels. This suggests new opportunity in IVF to reduce patients distress and improve quality of life, supporting the development of new research perspectives and new therapeutic strategy for LPS management.
Trial registration number: Protocol N° 63 of 5 February 2015

P-746 Serum vitamin D is not associated with ovarian reserve in infertile patients

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Study question: Is serum concentration of 25-hydroxyvitamin D [25(OH)D] associated with ovarian reserve in infertile patients?

Summary answer: Serum concentration of 25(OH)D is not associated with ovarian reserve in infertile patients.
What is known already: Vitamin D has been suggested to play an important role in female fertility which might be explained by the widespread distribution of vitamin D receptors in reproductive tissues. Vitamin D deficiency has been associated with significantly lower pregnancy rates in patients undergoing IVF treatment. An age dependent association between serum 25(OH)D and Anti-Müllerian Hormone (AMH) levels has been recently reported using, however, previous generation kits for AMH assessment, the accuracy of which has been criticized.
Study design, size, duration: This is a prospective, observational study, performed between June 2015 and December 2015, in 99 consecutive women, attending the outpatient infertility clinic of a University Hospital. Infertility was either idiopathic or due to female factor, male factor, or combination of both factors. In all patients, ovarian reserve was evaluated by assessment of baseline follicle stimulating hormone (FSH), serum AMH, as well as by ultrasound assessment of antral follicle count (AFC) and mean ovarian volume.
Participants/materials, setting, methods: AMH and 25(OH)D were measured using the automated Elecsys AMH and Elecsys Vitamin-D total assay (Roche-Diagnostics), respectively. The correlation of 25(OH)D with variables reflecting ovarian reserve was evaluated using Pearson's correlation coefficient. Multivariable logistic regression was used to assess the association between 25(OH)D and poor ovarian reserve, defined according to the Bologna criteria. The association of 25(OH)D with AMH, controlling for the effect of age, was assessed by generalized estimating equations (GEE).
Main results and the role of chance: The mean (95% CI) age of patients analysed was 39.0 years (38.1–39.9), with a body mass index of 25.5 (24.3–26.7) and infertility duration of 5.8 years (5.1–6.6). Baseline serum FSH and AMH levels were 10.8 (9.3–12.4) IU/mL and 1.6 (1.3–1.9) ng/mL, respectively. AFC and mean ovarian volume were 12.3 (10.5–14.0) and 6.5 (5.9–7.1) mL, respectively. Serum 25(OH)D levels were 21.4 (19.4–23.5) ng/mL. The proportions (95% CI) of patients with 25(OH)D levels <30 ng/mL and those with <10 ng/mL were 82.8% (73.9–89.7) and 11.1% (5.7–19.0), respectively. Poor ovarian reserve, according to the Bologna criteria, was present in 42.4% (32.5–52.8) of patients included.
No statistically significant correlation was observed between 25(OH)D levels and age (r=–0.037, p = 0.72), baseline FSH (r = –0.082, p = 0.42), AMH (r = 0.035, p = 0.73), AFC (r = 0.108, p = 0.29) or mean ovarian volume (r = –0.071, p = 0.48).
Logistic regression with dependent variable poor ovarian reserve, according to Bologna criteria, and independent variable 25(OH)D, as well as variables that were significantly associated with poor ovarian reserve in univariable logistic regression analysis (gravidity, cause of infertility and number of previous IVF cycles), showed no association of 25(OH)D with poor ovarian reserve (odds ratio: 1.002, 95% CI: 0.96–1.05). GEE analysis showed no association between 25(OH)D and AMH, controlling for the effect of age (p = 0.89).
Limitations, reasons for caution: A large proportion of patients in the current study fulfilled the Bologna criteria and this might have affected the results obtained. Moreover, sample size may have limited the establishment of a significant association between 25(OH)D and ovarian reserve.
Wider implications of the findings: Although vitamin D is considered to be associated with female fertility, this association does not appear to be mediated through ovarian reserve. If the current findings are confirmed, alternative explanations for the role of vitamin D in female fertility need to be explored.
Trial registration number: –

P-747 Recombinant luteinizing hormone (rLH) and recombinant follicle stimulating hormone (rFSH) for controlled ovarian stimulation in IVF/ICSI cycles

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Study question: Does adding recombinant LH (rLH) to recombinant FSH (rFSH) for controlled ovarian hyper stimulation (COS) increases pregnancy chances in women undergoing IVF/ICSI compared to rFSH alone?
Summary answer: Adding rLH to rFSH might increase pregnancy chances in women with poor ovarian response in agonist downregulated IVF/ICSI cycles.
What is known already: In 2006 a Cochrane review summarized studies on adding rLH to rFSH for COS in IVF/ICSI and described no evidence of a higher live birth rate (LBR). Since then more data has become available, in particular with respect to pregnancy-loss and poor ovarian response.
Study design, size, duration: We performed a systematic review and meta-analysis. We searched the following databases and trial registers to May 2015: CINAHL, the Gynaecology and Fertility Group Specialised Register, PsychInfo, CENTRAL, MEDLINE and EMBASE. There were no language restrictions. We included randomized controlled trials (RCTs) comparing rLH and rFSH to rFSH alone in IVF/ISCI cycles using either a GnRH agonist or antagonist for pituitary downregulation. Two review authors independently assessed trial quality and extracted data.
Participants/materials, setting, methods: The primary outcome was the live birth rate (LBR) and the primary safety outcome was the incidence of ovarian hyperstimulation syndrome (OHSS). We combined data to calculate odds ratios (ORs) and 95% confidence intervals (CIs). Statistical heterogeneity was assessed using the I^2 statistic. We assessed the overall quality of the evidence for the main comparison using GRADE methods.
Main results and the role of chance: We included 34 RCTs consisting of 7979 women. We found no difference in LBR between rLH and rFSH (rLH + rFSH) and rFSH alone using GnRH agonists (OR 1.74, 95% CI 0.81–3.77; n = 474; RCTs = 5; I^2 = 53%) nor using GnRH antagonists (OR 0.94, 95% CI 0.48–1.85; n = 240; RCTs = 1) in all women. The ongoing pregnancies (OR 1.27, 95% CI 1.02–1.57; n = 1980; RCTs = 14; I^2 = 17%) and clinical pregnancies (OR 1.21, 95% CI 1.05 to 1.40, RCTs = 19, n = 4189, I^2 = 34%) were significantly higher in women treated with rLH + rFSH compared to rFSH alone using GnRH agonists. We found no difference in ongoing (OR 1.08, 95% CI 0.82–1.43; n = 1149; RCTs = 7; I^2 = 0%) or clinical (OR 0.90, 95% CI 0.65–1.24, RCTs = 4, n = 776, I^2 = 0%) pregnancies between rLH + rFSH in women using GnRH antagonists.

We found lower or respectively no difference in OHSS following COS with rLH + rFSH compared to rFSH alone using GnRH agonists (OR 0.16, 95% CI 0.03–0.88, RCTs = 4, n = 1418, I^2 = 0%) GnRH antagonists (OR 0.80, 95% CI 0.21–3.00, RCTs = 2, n = 760).

In a sub analysis of women with poor ovarian response we found that adding rLH to rFSH in GnRH agonist downregulated IVF/ICSI resulted in higher ongoing pregnancy rate compared to rFSH alone (OR 2.06, 95% CI 1.20–3.53; n = 276; RCT's = 3; I^2 = 0%) but not in women with normal response (OR 1.17, 95% CI 0.91–1.51; n = 1349; RCTs = 7; I^2 = 34%).
Limitations, reasons for caution: The overall quality of the included studies was low due to poor reporting of study methods and lack of precision in the findings for most of the GRADE-specific outcomes.
Wider implications of the findings: The evidence suggests that there might be a beneficial effect of rLH + rFSH in GnRH agonist down regulated IVF/ICSI cycles in poor-responders. The risk of OHSS is not increased by rLH. IPD meta-analysis is required to better estimate the effect of poor response on the effectiveness of rLH + FSH.
Trial registration number: NA.

P-748 The preventive effect of resveratrol in cisplatin-induced ovarian damage

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Study question: Is there a preventive effect of resveratrol in cisplatin (CP)-induced ovarian damage?
Summary answer: Resveratrol may be effective in maintenance of primordial, primary and tertiary follicle levels in cisplatin-induced ovarian damage.

What is known already: Some cancers develop in women of reproductive age. Every year thousands of women receive chemotherapy for cancer treatment; hence some women are faced with a reduction in fertility from minimal to complete ovarian failure. Therefore, many of these women may be needed fertility preservation options. Resveratrol, a natural biochemical found in red wine and certain foods, that has antioxidant properties. Increased levels of reactive oxygen species may play an important role in the ovarian aging process. In this study we aimed to evaluate whether resveratrol has protective effects on cisplatin-induced ovarian damage.
Study design, size, duration: Experimental study.
Participants/materials, setting, methods: 28 female Wistar–albino rats were divided into 4-equal groups. Group1 was defined as sham group. During 21-day-study period, 5 mg/kg/day resveratrol was given to group2, 25 mg/kg/day resveratrol to group3, 1 ml/kg/day saline to group4. Three blood samples were taken of all groups on basal, 15 and 21 days of treatment, and anti-Mullerian hormone (AMH) levels were measured. All rats were oophorectomized 1 week after the chemotherapeutic agent and ovarian follicles were counted.
Main results and the role of chance: No significant difference was found in AMH levels of groups according to the follow up time after Bonferroni correction ($p > 0.0125$). In group 2 the number of primordial follicles was statistically higher than other groups ($p < 0.05$). The number of primordial follicles in group 3 was statistically lower than group 1 and 2; higher than group 4 ($p < 0.05$). In group 4; number of primordial follicles was statistically lower than the other groups (according to group 1 $p < 0.001$; to group 2 and 3 $p < 0.05$). In group 2; the number of primary follicles was higher than the other groups ($p < 0.05$). In group 3 the number of primary follicles was statistically significantly lower than group 2, higher than group 4 ($p < 0.05$). In group 4; levels of primary follicles were lower than the others (according to group 1 $p < 0.001$; to group 2 and 3 $p < 0.05$). In group 2 and 3; levels of tertiary follicles were higher than group 4, lower than group 1 ($p < 0.05$). Tertiary follicles were the lowest levels in group 4 (according to group 1 $p < 0.001$; to group 2 and 3 $p < 0.05$).
Limitations, reasons for caution: (i) Limited number of subjects, (ii) the implementation of a single dose of cisplatin, (iii) short study period, (iv) lack of knowledge of the optimal dose of resveratrol.
Wider implications of the findings: Resveratrol may be effective in maintenance of primordial, primary and tertiary follicle levels in cisplatin-induced ovarian damage. There is a need for further studies to investigate the effects of resveratrol on human ovaries.
Trial registration number: Ankara Training and Research Hospital Animal Experiments Local Ethics Committee, date and decision number: 04 June 2015/0024.

P-749 The role of PI3-K signalling pathway in insulin actions on testosterone synthesis in peripheral adipose tissue of women with and without polycystic ovarian syndrome

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Study question: Is there a role for PI3-K signalling pathway in insulin regulation of testosterone synthesis in peripheral adipose tissue of women with and without polycystic ovarian syndrome (PCOS)?
Summary answer: PI3-K signalling pathway does not seem to be involved in insulin regulation of testosterone synthesis in peripheral adipose tissue of women with or without PCOS.
What is known already: The positive correlation between the level of circulating androgens, insulin resistance and central obesity is well established. In addition, recent *in vitro* studies have provided evidence of excess androgen synthesis in peripheral adipose tissue of women with PCOS, which could be a potential source of hyperandrogenism. Further research showed insulin to up-regulate the expression of androgen synthesis enzymes (CYP 17 and AKR1C3) as well as testosterone synthesis in peripheral adipocytes of normal but not PCOS women. The underlying mechanisms and the exact signalling pathway involved in the insulin actions on steroidogenesis in adipose tissue of normal women remains to be determined.
Study design, size, duration: This was a laboratory based study involving an *in vitro* differentiated serum free monolayer cell culture of adipocytes harvested from subcutaneous adipose tissue obtained during gynaecological surgery from women with ($n = 5$) and without ($n = 5$) PCOS. All participants were of reproductive age (20–45) with a BMI of 20–35 kg/m^2.
Participants/materials, setting, methods: Pre-adipocytes were isolated from the adipose tissue samples and differentiated to mature adipocytes, which were cultured in FCS-free medium. Recombinant insulin ± LH ± PI3-k inhibitor (LY294002) was added to the cell culture at different concentrations. The supernatant was collected for testosterone measurement before and after treatment with insulin ± LH ± LY294002 using enzyme-linked immunosorbent assay (ELISA).
Main results and the role of chance: Testosterone concentration in the supernatant of untreated cultured PCOS adipocytes (mean ± sem, 129.3 ± 2.5 pg/ml) was significantly ($P < 0.0001$) higher than that (33.7 ± 4.6 pg/ml) of non-PCOS adipocytes. Insulin addition in different concentrations (1 nM/l, 10 nM/l, 100 nM/l) caused a significant increase of testosterone concentrations (94.1 ± 7.1; 118.2 ± 18.2, 200.0 ± 7.3 pg/ml respectively) in the supernatant of cultured non-PCOS adipocytes but not the PCOS adipocytes (118.1 ± 1.8, 90.5 ± 6.4, 89.3 ± 7.6 pg/ml respectively). The increase of testosterone levels in the non-PCOS adipocyte culture supernatant followed a dose dependant fashion. The magnitude of increase in testosterone concentrations in non-PCOS adipocyte culture supernatant was markedly increased when LH was added to insulin. Adding PI3-K inhibitor (LY294002, 1010 ng/ml) to insulin did not change the magnitude of insulin stimulatory effects on testosterone concentrations in non-PCOS adipocyte culture supernatant (Insulin 1 nM/l + LY, testosterone 91.8 ± 2.6; Insulin 10 nM/l + LY, testosterone 119.6 ± 19.4 pg/ml; Insulin 100 nM/l + LY, testosterone 175.1 ± 15.4 pg/l).
Limitations, reasons for caution: The number of biopsies was relatively small in this study and the results will therefore need to be verified by further larger studies.
Wider implications of the findings: Peripheral adipose tissue testosterone levels seem markedly elevated in PCOS women. This supports the hypothesis that excess adipose tissue androgen synthesis plays an important role in PCOS pathogenesis. Insulin±LH seem to play a role in the increased androgen synthesis in adipose tissue, but not through the PI3-K signalling pathway.
Trial registration number: N/A.

P-750 Recombinant vs. urinary human chorionic gonadotrophin (hCG) for triggering final oocyte maturation in patients at risk for ovarian hyperstimulation syndrome OHSS: a randomized clinical trial

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Study question: Is the number of metaphase-II (MII) oocytes associated with the type of hCG used for triggering final oocyte maturation in patients at risk for OHSS?
Summary answer: The number of MII oocytes is not associated with the type of hCG used for triggering final oocyte maturation in patients at risk for OHSS.
What is known already: Urinary hCG (u-hCG) and recombinant hCG (rec-hCG) are widely used for triggering final oocyte maturation in patients undergoing ovarian stimulation for *in vitro* fertilization (IVF). Previous studies comparing various doses of u-hCG and rec-hCG have been conducted in normal responders, undergoing long down-regulation protocols. The meta-analysis of these studies suggests that the number of oocytes retrieved, severe OHSS and ongoing/live birth rates are similar between the u-hCG and rec-hCG groups. However, information on the efficacy and safety, of rec-hCG and u-hCG in patients at risk for ovarian hyperstimulation syndrome (OHSS), using GnRH antagonist and recombinant FSH (rec-FSH) is currently not available.
Study design, size, duration: One-hundred patients, undergoing ICSI between 11/2013 and 11/2015 in a single ART center, were randomized to receive either rec-hCG or u-hCG, provided that ≥14 and <24 follicles >11 mm were present at ultrasound on the day of triggering final oocyte maturation. On that day, a study nurse performed random allocation, using a computer-generated randomization list. Based on previously published data, the study was powered to detect a difference of 2.3 MII oocytes between groups at $a = 0.05$ and $b = 0.20$.

Participants/materials, setting, methods: Patients (aged 18–39) underwent ovarian stimulation with recFSH and gonadotrophin releasing hormone (GnRH) antagonists. Following randomization to rec-hCG or u-hCG and oocyte retrieval, fertilization was performed by intracytoplasmic sperm injection (ICSI). The primary outcome measure was the number of MII-oocytes, assessed by an embryologist blinded to the type of the triggering signal. Secondary outcome measures included number of oocytes retrieved, number of 2PN oocytes, ongoing pregnancy rates and incidence of severe early or late OHSS.

Main results and the role of chance: No significant differences were observed between the rec-hCG and u-hCG groups regarding female age (years) (33.7, 95% CI: 32.6–34.8 vs. 33.6, 95% CI: 32.6–34.6, respectively; $p = 0.916$), BMI (Kg/m^2) (23.1, 95% CI: 21.9–24.4 vs. 24.6, 95% CI: 23.3–25.9, respectively; $p = 0.102$), or basal FSH (IU/L) (7.3, 95% CI: 6.87.8 vs. 6.9, 95% CI: 6.5–7.4, respectively; $p = 0.324$). Moreover, no significant differences were observed regarding duration of stimulation (days) (10.2, 95% CI: 9.8–10.6 vs. 9.8, 95% CI: 9.4–10.2, respectively; $p = 0.141$) and total units of gonadotrophins required (1742, 95% CI: 1644–1841 vs. 1708, 95% CI: 1579–1837, respectively; $p = 0.669$).

The mean number of MII-oocytes, was not different between the rec-hCG and the u-hCG groups (12.3, 95% CI: 11.4–13.2 vs. 13.5, 95% CI: 12.6–14.3, respectively; $p = 0.056$).

In addition, rec-hCG and u-hCG groups did not differ in the proportion (%) of mature oocytes retrieved (76.4, 95% CI: 72.3–80.6 vs. 80.2, 95% CI: 76.1–84.3, respectively; $p = 0.205$), in the number of oocytes retrieved (16.1, 95% CI: 15.4–16.8 vs. 16.8, 95% CI: 16.1–17.5, respectively; $p = 0.188$) and in the number of 2PN oocytes (9.1, 95% CI: 8.1–10.0 vs. 9.6, 95% CI: 8.6–10.5, respectively; $p = 0.449$). Finally, the two groups did not differ in ongoing pregnancy rates (23/50 = 46%, 95% CI: 32.1%–60.5% vs. 30/50 = 60%, 95% CI: 45.2%–73.3% respectively; $p = 0.229$). No cases of severe early or late OHSS were observed in either group (95% CI: 0–7.1%).

Limitations, reasons for caution: The patients included in the current study opted to undergo fresh embryo transfer despite extensive counseling regarding the associated risk of severe OHSS and the availability of alternative methods to trigger final oocyte maturation, such as the use of GnRH agonist triggering combined with embryo cryopreservation.

Wider implications of the findings: Both rec-hCG or u-hCG, used for triggering final oocyte maturation, in patients at risk for OHSS appear to have a similar reproductive outcome and OHSS incidence. However, the ease of use, the higher degree of purity and specificity characterizing rec-hCG should be weighted against the lower cost of u-hCG.

Trial registration number: NCT00415766.

P-751 Interventions to improve ART outcome in PCOS patients: a systematic review and meta-analysis

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Study question: Are there any effective interventions which improve ART treatment in women with polycystic ovary syndrome (PCOS)?

Summary answer**:** Low quality evidence suggests benefit of antagonist protocols and metformin Supplementation. Ovulation induction and estradiol is equally effective for endometrial preparation before FET.

What is known already: Recent developments have seen the introduction of various measures to reduce the risks of OHSS and cycle cancellation and to improve oocyte quality. It remains unclear, how all these new approaches have impacted on ART outcomes in PCOS women.

Study design, size, duration: We performed a systematic review and meta-analysis. A comprehensive literature search of the standard medical databases was performed. The last electronic search was run in July 2015.

Participants/materials, setting, methods: Randomized controlled trials evaluating interventions aiming to improve the effectiveness or to reduce complications of ART in women diagnosed with PCOS were included. Data from the included studies was extracted independently by two authors using a predefined pro-forma. Statistical analysis was performed using RevMan version 5.3 (The Nordic Cochrane Centre, Copenhagen, Denmark) and MedCalc version 12.7 (MedCalc Software bvba, Ostend, Belgium).

Main results and the role of chance: We screened 1,021, completely assessed 173 records, and finally included 66 studies in the quantitative analysis. Many different interventions were assessed, however overall quality of studies was low. We observed moderate quality evidence that there is no clinically relevant difference on live birth/ongoing pregnancy, or clinical pregnancy when comparing antagonist and agonist protocols for controlled ovarian stimulation. Additionally, we found low quality evidence that metformin improves live birth/ongoing pregnancy and clinical pregnancy rates. We further found low quality evidence that there is no clinically relevant difference on live birth/ongoing pregnancy and clinical pregnancy when comparing human menopausal gonadotropin (hMG) for inducing ovulation and artificial preparation with estradiol valerate for endometrial preparation for frozen embryo transfer. Low quality evidence suggests that mannitol and antagonist protocols reduce ovarian hyperstimulation syndrome (OHSS).

Limitations, reasons for caution: Limitations included methodological quality, marginal variability of investigated interventions and outcomes.

Wider implications of the findings: Further investigation is recommended. To facilitate metaanalysis, future research should be done using outcome measures consistent with those in the studies included here.

Trial registration number: PROSPERO – CRD42014007304

P-752 Are polyfluoroalkyl chemicals associated with raised levels of serum testosterone in women with and without Polycystic Ovary Syndrome, undergoing controlled ovarian hyperstimulation IVF cycle

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Study question: Could Polyfluoroalkyl agents(PFAAs) be an environmental trigger for abnormal testosterone production in women with and without Polycystic Ovary Syndrome?

Summary answer: PFAAs have significant correlation with serum testosterone levels in both women with and without PCOS.

What is known already: PFAAs are formed by degradation of polyfluorinated compounds used in many consumer products such as in the paper and textile industry. The three most abundant PFAAs are perfluoro octanoic acid (PFOA), perfluorooctane sulfonic acid (PFOS), and perfluorohexane sulfonic acid (PFHxS), with half-lives to be 3.8, 5.4, and 8.5 years, respectively.

There is minimal literature on PFAAs, but animal studies have demonstrated that exposure to PFOS and PFOA may affect the integrate balance of sex hormones and pregnancy loss in animals. Exposure of male rats to PFOA can reduce testosterone levels, resulting in hyperplasia of the Leydig cells within the testis.

Study design, size, duration: This prospective cohort study was undertaken over a 6-month period in the Hull IVF Unit, UK. PCOS women were selected using the revised 2003 criteria from the Rotterdam ESHRE/ASRM sponsored PCOS consensus workshop group. No patients were lost to follow-up. Statistical analysis was performed to assess for potential associations with the levels of serum contamination of these endocrine disrupting agents (EDAs), pregnancy rates and endocrine profiles of the subjects.

Participants/materials, setting, methods: Fasting serum samples were collected from 59 (29 PCOS and 30 controls) women undergoing IVF/ICSI. The serum samples were analysed using Liquid chromatography-tandem mass spectrometry to measure the concentration of the polyfluoroalkyl congeners. Testosterone is measured by stable isotope dilution chromatography-tandem mass spectrometry. All patients underwent a standard IVF antagonist protocol, embryo transfers were performed on day 3 or ideally at day 5 (blastocyst) to give the best chance for implantation.

Main results and the role of chance: In this present study PFOS, PFOA, PFHxS and perfluorononanoic acid (PFNA) were detected in 100% of the serum samples and perfluorononanoic acid (PFDA) was detected in 76% of samples. PFOS was the PFAA detected in the highest concentration in both the PCOS and control groups, 4.11 ± 1.62ng/ml and 3.33 ± 1.05ng/ml respectively. PFOS concentration was significantly higher in the PCOS group compared to the controls, $p = 0.03$. The levels of PFOS in this study were significantly lower than recent literature, confirming that levels of EDCs continue to decline following the ban of these chemicals. The PFAAs had significant positive correlations with testosterone in the overall patient population and in both the control and PCOS subgroups. In the 59 patients overall, PFOS and PFDA had very significant positive correlations with testosterone ($r = 0.411$, $p = 0.001$, and $r = 0.393$, $p = 0.002$ respectively), with PFHxS, PFOA and PFNA having significant positive correlations at the $p = 0.05$ level. In the control group PFOS and PFOA maintained a significant positive correlation ($p = 0.02$ and $p = 0.04$ respectively) and in the PCOS group PFOS and PFDA maintained a significant positive correlation ($p = 0.03$ and $P = 0.02$ respectively). There was no correlation between the levels of EDAs and pregnancy rates in either the PCOS and control groups.
Limitations, reasons for caution: Studies have demonstrated associations with EDAs and adverse reproductive effects, however no standard validated test can assess potential exposure to EDAs and possible adverse reproductive outcomes. This small study has demonstrated a possible endocrine disrupting effect of PFAAs and testosterone but larger studies are needed to support these findings.
Wider implications of the findings: PFAAs may effect the androgen pathway in both women with and without PCOS, but the higher levels of PFOS in the PCOS group may demonstrate that this population of women may be more prone to the effects of this group of EDAs.
Trial registration number: Not applicable

P-753 Altered expression of Wnt/beta-catenin signaling pathway in endometrium of women with polycystic ovarian syndrome (PCOS)

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Study question: Is Wnt/beta-catenin signaling pathway abnormally expressed in the endometrium of women with polycystic ovarian syndrome (PCOS)?
Summary answer: The endometrium of women with PCOS exhibit altered beta-catenin expression compared to fertile cycling women
What is known already: The Wnt/beta-catenin signaling contributes to normal endometrial proliferation and secretory transformation driven by ovarian steroids during menstrual cycle. Unopposed estrogen action may lead to constitutive Wnt/beta-catenin activation triggering endometrial hyperplasia and cancer. Risk factors for endometrial hyperplasia and cancer includes PCOS.
Study design, size, duration: Retrospective observational study was designed to compare the expression of beta-catenin in endometrial samples of 32 fertile women with regular menses and infertile women with PCOS diagnosed according to Rotterdam criteria obtained during 2012–2014 at university affiliated institution in Santiago Chile.
Participants/materials, setting, methods: Endometrial biopsy was obtained during proliferative phase. Institutional Ethics Board approved the protocol. Beta-catenin was localized by immunohistochemistry. Histologic score (Hscore) was assigned for immunostaining. Protein abundance and mRNA levels was determined by western blot and qRT-PCR in endometrial epithelial cells harvested by laser capture microdissection (LCM). Non parametric Mann–Whitney test was employed for comparison of numerical variables.
Main results and the role of chance: Cytoplasmic and nuclear beta-catenin was localized in endometrial cells. According to Hscore high beta-catenin expression was found in proliferative endometrium from women with PCOS compared to fertile cycling women 3.6 ± 0.2 vs. 2.1 ± 0.2 respectively. Western blot for beta-catenin corroborated high protein abundance in PCOS samples. The mRNA levels in epithelial cells dissected by LCM confirmed high beta-catenin expression in PCOS samples 2.7 ± 0.1 vs. 1.4 ± 0.1 respectively.
Limitations, reasons for caution: This is a retrospective study including limited number of samples.
Wider implications of the findings: The altered Wnt/beta-catenin pathway found in this study may represent the early molecular changes of endometrial abnormalities associated with PCOS.
Trial registration number: No required

POSTER VIEWING SESSION

REPRODUCTIVE EPIDEMIOLOGY, SOCIO-CULTURAL ASPECTS AND HEALTH ECONOMY

P-754 Alcohol consumption and live birth among fertility patients: a nationwide register-based cohort study

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Study question: Does preconceptional female and male alcohol consumption influence the probability of achieving a live birth after assisted reproductive technology (ART) treatment?
Summary answer: Male alcohol consumption seems to negatively affect probability of a live birth after ART. Female alcohol consumption was not associated with odds of live birth.
What is known already: Previous studies have shown inconsistent results. One study found no association between female alcohol consumption and live birth, while another found that women consuming at least 4 drinks/week, had 16% lower odds of achieving a live birth compared to women consuming less. An increase in paternal alcohol consumption of one drink/day increased the risk of not achieving a live birth by 3.14 times, while, in another study, no significant effect of male intake was seen. Odds of live birth were 21% lower among couples where both partners consumed at least 4 drinks/week, compared to couples where both spouses consumed less.
Study design, size, duration: A Danish national, register-based prospective cohort study; A total of 29,834 treatment cycles performed on 12,981 women undergoing fertility treatment in the period from January 1, 2006 to September 30, 2010, were included in the study. Collection of outcome was continued till June 30, 2011.
Participants/materials, setting, methods: All Danish women undergoing at least one treatment cycle with autologous oocytes in the study period were eligible for this study. Information on preconceptional alcohol consumption was extracted from the *in vitro* fertilization (IVF) register and linked to registries containing information on all births and abortions. We performed multilevel logistic regression analyses to estimate odds of live birth after ART under consideration of the non-equal rate of success between two different individuals.
Main results and the role of chance: In total, 12,981 women and their partners went through 29,834 treatment cycles. For female abstainers and heavy consumers (>7 drinks/week) the live birth rate was 22.4% and 20.4%, respectively. Concerning men, 22.6% of cycles led to a live birth, when the man was abstinent, compared to 20.2% of cycles in male heavy consumers (>14 drinks/week). Overall, results from statistical analyses did not reach significance, but interesting tendencies were seen. For men, a tendency of decreasing odds with increasing alcohol consumption was observed (OR 0.97 95% CI 0.73–1.30, 0.89 95% CI 0.68–1.15 and 0.74 95% CI 0.44–1.25 for light (1–2 drinks/week), moderate (3–14 drinks/week) and heavy consumers respectively), when comparing to male abstainers. From the trend analysis, a decrease of one percent (OR for trend 0.99; 95% CI 0.97–1.01) was observed with every one-unit increase in weekly alcohol consumption. Female alcohol consumption did not seem to affect odds of achieving a live birth (OR for trend 1.00; 95% CI 0.99–1.01). Combined results on couples were inconclusive.
Limitations, reasons for caution: Alcohol consumption was self-reported, which can cause misclassification due to information bias. However, applying different sensitivity levels did not change our estimates markedly. We excluded 11,205 cycles (24.7% of baseline) due to missing information on female alcohol consumption, but we do not expect non-response to be related to alcohol consumption.
Wider implications of the findings: In accordance with previous findings, our results suggest that preconceptional male alcohol consumption is negatively associated with the ability to achieve a live birth after ART treatment. More focus on both partners' alcohol consumption may be beneficial in the preconceptional care of couples seeking fertility treatment.
Trial registration number: n/a

P-755 World trends in Assisted Reproductive Technology (ART) 2004–2013

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Study question: How have ART outcomes evolved around the world over the last decade?
Summary answer: International ART practices are characterized by highly significant outcome differences between regions.
What is known already: ART has undergone considerable changes over the last decade, with different regions of the world at times going in different directions.
Study design, size, duration: Based on data retrieved from national ART registries, we report how changes in clinical practice between 2004 and 2013 have impacted outcomes in Australia and New Zealand, Canada, Continental Europe, the UK, Japan, Latin America, and the United States (US).
Participants/materials, setting, methods: The data reflect 6,589,581 total ART cycles utilizing both fresh and previously cryopreserved embryos from autologous oocytes that resulted in 1,335,615 live births.
Main results and the role of chance: We observed a worldwide increase in the utilization of single embryo transfer (SET) and of previously frozen embryos. In 2012–2013, fresh cycle live birth rates were highest in the U.S. (29%) and the lowest in Japan (5%). While gradual improvements in live birth rates were observed in most regions, some regions exhibited either no change or even declines. A significant decline in fresh ART live birth rates were most apparent in Japan and Canada. Declining fresh cycle live birth rates in Japan were partially offset by improved rates with frozen cycles. This shift coincided with introduction of mild ovarian stimulation ("mini IVF"), embryo banking and blastocyst stage elective SET protocols. Concomitantly, fresh ART cycle starts in Japan tripled. Declining fresh cycle live birth rates in Canada coincided with implementation of an SET policy in Quebec.
Limitations, reasons for caution: National data sets vary greatly in data collection and quality. They, therefore, are not necessarily comparable. Observed associations with changes in regional IVF practice do not necessarily denote causations.
Wider implications of the findings: Trends suggest that some newly introduced ART practices may have negatively affected outcomes. New ART practices should be introduced to general clinical practice more cautiously. An international consensus on the definition of ART success may be required before attempts to resolve discrepancies in worldwide ART outcomes can be fully realized.
Trial registration number: N/A

P-756 Credibility test in meta-analysis: a support for clinical decision

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Study question: This study proposes a simple test to analyse the strength of meta-analysis results.
Summary answer: The credibility test is an important tool for quality control of the results of a meta-analysis.
What is known already: Evidence-based medicine has elevated meta-analyses to the status of key parameters in medical decisions. Every year, over 6,000 meta-analyses are published in medical journals and submitted to the appreciation of physicians, who then must analyse their conclusions and use them to support medical decisions. ART does not escape this rule, and meta-analyses concerning topics with controversy (PGS, GnRH agonists vs. GnRH antagonists, etc.) are frequently published. However, some meta-analyses reach conclusions that periodically change with the addition of new prospective randomized trials.
Study design, size, duration: Two meta-analyses were chosen for the application of the credibility test.

1. Anticoagulant prophylaxis (Dentali et al., 2007) for the prevention of pulmonary embolism; results are in favour of the use of anticoagulants.
2. Endometrial injury (Nastry et al., 2015). An analysis of clinical pregnancy in women with ≥2 previous embryo transfers; results are in favour of the use of endometrial injury.

Participants/materials, setting, methods: Performing the credibility test consists of removing at least 1 study from a meta-analysis and observing whether the original results remain. Interpretation: Credibility Present: the removal of one or more articles does not affect the conclusion of the meta-analysis. Credibility Absent: the removal of one article affected the conclusion of the meta-analysis. Data were analysed using StatsDirect. Data were expressed as in the original meta-analysis. Heterogeneity was evaluated using Cochran's Q and I^2 tests.
Main results and the role of chance: Meta-analysis I: credibility is present. Even after the random removal of two trials, the result continued to significantly favour the use of anticoagulants for the prevention of pulmonary thromboembolism. The result has credibility for clinical application. Meta-analysis II: credibility is absent. The result has no credibility for clinical application. After the random removal of only one trial, the result changed and was no longer statistically significant.

P-757 The association between Hepatitis B virus infection and the underlying cause of infertility – A study in an endemic region

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Study question: Is hepatitis B virus infection associated with a specific underlying cause of infertility?
Summary answer: Hepatitis B virus infection was associated with secondary infertility due to tubal and uterine factors.
What is known already: Screening for hepatitis B virus infection is performed routinely in the workup investigation in infertile couples. Positive screening in the male partner has been associated with poor sperm quality. There are conflicting reports on the impact of infection in either partner on the outcome of in-vitro fertilization treatment. However, it is unknown whether infection in either partner is associated with a specific underlying cause of infertility. In this study, we explored the relationship between infection in either partner and the underlying cause of infertility in Hong Kong, where the infection is endemic with reported prevalence of 10% in obstetric population.
Study design, size, duration: This is a retrospective observational study conducted among consecutive infertility couples managed in the Assisted Reproductive Unit, the tertiary referral centre affiliated with the Department of Obstetrics and Gynecology, The Chinese University of Hong Kong, during the period January to October 2015.
Participants/materials, setting, methods: There were 739 couples managed during the study period. The causes of infertility were classified into tubal factor, pelvic adhesion, endometriosis, uterine factor, male, anovulation, polycystic ovarian syndrome, hyperprolactinaema, immunological, coital and unexplained factors. The hepatitis B viral serostatus for the couple were checked upon referral and within 2 years of the treatment cycles.
Main results and the role of chance: Husband of female hepatitis B carrier is 3.15 times risk of being hepatitis B carrier (18.4% vs. 6.7% [$P = 0.003$; OR 3.15, CI 1.440–6.889]). In the female subjects, hepatitis B carriage was more likely to be associated with secondary infertility (63.0% vs. 45.2% [$P = 0.014$; OR 0.479, CI 0.263–0.873]); and with tubal factor (71.4% vs. 42.8% [$P < 0.000$; OR 3.347, CI 1.769–6.335]) and uterine factor (14.6% vs. 3.0% in non-hepatitis carriers [$P < 0.000$; OR 5.431, CI 2.182–13.515]), but was less likely to have unexplained infertility (2.0% vs. 13.8% [$P = 0.018$; OR 0.130, CI 0.018–0.957]). There was no significant association with the other causes that included pelvic adhesion, endometriosis, anovulation, polycystic

ovarian syndrome, hyperprolactinaema, immunological, coital dysfunction, or male factor. In the male subjects, hepatitis B carriage was also associated with tubal factor in female partners (60.0% vs. 43.4% [$P = 0.017$; OR 1.955, CI 1.116–3.423]), but there was no significant association with the other factors, including male factor or coital dysfunction.

Limitations, reasons for caution: Hepatitis B virus could have served as surrogate for other infections in its association with tubal factors of infertility. Although sexually transmitted diseases were screened routinely by culture of endocervical swab, infections such as chlamydial infection could be missed due to the low yield of this method.

Wider implications of the findings: Hepatitis B carriage in infertile women increased the likelihood of tubal and uterine factors but not other infertility factors. The exact underlying reason is unclear but it may alter the immunological response to tubal-peritoneal infections. A positive screening result should be an indication to vigilantly rule out any sexually-transmitted infections.

Trial registration number: Nil

P-758 Does rationing fertility treatment lead to better success rates? A population-based comparative analysis of Australian and New Zealand assisted reproductive technology cycles

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Study question: Are the differences in the success rates of assisted reproductive technology (ART) cycles between Australia and New Zealand explained by different funding approaches?

Summary answer: Different funding approaches may have resulted in healthier women undergoing treatment and may explain the improved pregnancy outcomes in New Zealand couples having fertility treatments.

What is known already: The difference in funding arrangements of ART procedures between Australia and New Zealand has meant that Australian women had greater access to treatment compared to women in New Zealand. In 2010, we first reported on the impact of the different funding arrangements on access and outcomes in Australia and New Zealand from the years 2004 to 2007. In that report, the rates of live delivery following fresh ART cycles were significantly higher in New Zealand than in Australia in all age groups, in spite of higher proportions of single embryo transfer (SET) in New Zealand.

Study design, size, duration: A comparative analysis of the treatment, pregnancy and perinatal outcomes of autologous ART cycles in Australia and New Zealand from 2010 to 2013 in the context of policy where New Zealand has specified criteria in order to access public funded fertility treatment and Australia does not.

Participants/materials, setting, methods: 162,952 autologous fresh cycles and 93,376 autologous thaw cycles from Australian and New Zealand Assisted Reproduction Database (ANZARD). ANZARD data are collected annually, in de-identified format, from all fertility centres in Australia and New Zealand. ANZARD includes information about the demographic characteristics of patients undergoing treatment, the types of ART procedures performed, and information on pregnancy and birth outcomes.

Main results and the role of chance: For all age groups, the live birth rates following fresh embryo transfer cycles were significantly higher in New Zealand (23.7%) than in Australia (16.5%) a difference of 8.2% ($p < 0.01$). The crude rate of live delivery per initiated fresh cycles for all women was 1.43 times higher in New Zealand than in Australia (RR 1.43, 95% CI 1.38–1.48). These differences in outcomes persisted in all age groups. The difference in live delivery between New Zealand and Australia was also present in all age groups in thaw cycles although the difference was smaller (23.3% vs. 19.8%, $p < 0.01$). Overall rates of single embryo transfer (SET) are similar between Australia and New Zealand, but significant differences are seen within age categories, with New Zealand utilizing more SET in women aged <35 (90.7% vs. 82.5%, $p < 0.01$) and less SET in women aged >40 compared to Australia (33.8% vs. 53.3%, $p < 0.01$). In agreement with the SET rates, for women aged less than 35 years, there were increased rates of singletons in New Zealand compared with Australia (95.4% vs. 93.3%, $p < 0.01$). Blastocyst transfer rates were also higher in Australia (53.3%) than New Zealand (39.1%, $p < 0.01$) as was intracytoplasmic sperm injection rates (65.3% and 56.7% $p < 0.01$).

Limitations, reasons for caution: Data was not available on length of infertility, body mass index or number of treatment cycles. There was no data on how each cycle is funded in New Zealand. 13.5% of Australian data did not report a cause of infertility. There was a higher proportion with unexplained infertility in Australia.

Wider implications of the findings: Restricting access to treatment may limit utilization but may also select an infertile but healthier population (not obese, non smokers). The purpose of the New Zealand criteria is to treat women with a low likelihood of natural pregnancy and also avoid pregnancies in women at risk of poor perinatal outcomes.

Trial registration number: Not required

P-759 Lethal consequences of infertility on lives of poor women surviving in developing countries like Pakistan

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Study question: Questionnaire was given, where age, duration of infertility, socioeconomic status, known previous ailment (medical or psychological), permanent contact numbers, social and personal status of couple and agreement for future liaison.

Summary answer: Females belonging to poor to average socioeconomic status with age between 33 and 38 years were selected in this study group.

What is known already: Being infertile in developing countries like Pakistan is social crime for women, leading to medical, psychiatric and sometimes surgical problems. Severity is directly related to duration of infertility, extent of poverty and mean age of women. In this study we aimed to investigate that what are the ultimate consequences of infertility on these poor helpless dependent women of Pakistan. Since women of Pakistan are already bearing the crises of male dominating society, so they are prone to be victimized easily resulting in severe medical and psychological distress.

Study design, size, duration: RCT conducted on 252 female patients in Andrology institute of Pakistan (STAR ICSI), between 1st August 2010 and 31st July 2012. A questionnaire was given to gather information about sociodermographic characteristics, and was completed by the individual. Later on follow up was done during 1st January 2014 to 31st December 2015, to see further consequences on infertile women.

Participants/materials, setting, methods: Total 252 females belonging to poor to average socioeconomical status were selected in this study group. Mean age of females was between 33 and 38 years. Duration of infertility was between 5 and 10 years. Patients with known medical disorders were excluded in this group. A bond of agreement was signed by patients, where they agreed to provide future informations about their personal marital lives.

Main results and the role of chance: Among 252 females, 73.41% of them were having primary infertility. During the follow up of consecutive 2 years, 69 patients conceived. It means 27.3% became pregnant. Among remaining 183 Infertile patients, 22 females experienced severe social disaster resulting in the form of divorce. It means 12.2% of females were divorced just because of their inability to conceive. 47 patients are still living separately from their husbands just because of this allegation that they cannot procreate. It means 25.6% of patients suffered from severe social crises. 73 patients suffered from some psychiatric problems for which they have to seek advise and medication from psychiatrist. It means 39.8% of infertile patients underwent some psychiatric problems just because of their stigma of infertility. 27 patients experienced disastrous medical and surgical complications due to maltreatment by dais and quakes. It means 14.7% of patients underwent serious complications of maltreatment. 4 patients died during these 2 years. According to some people of their premises they have

committed suicide (no legal record). 10 patients are still seeking medical help and ready to go for ART. It means 5.4% of patients are still on right pathway and not loosing their hopes.
Limitations, reasons for caution: Developing country like Pakistan who is unable to cope with stigma of population explosion, cannot offer any help to poor infertile dependent female, who is unable to handle this issues alone in this male dominating society, resulting in bad medical and psychological impacts in their lives.
Wider implications of the findings: Although this study has limitations regarding sample size and duration but still this small study may help to create a revolution in the field of infertility, for poor masses in developing countries and may create light in the dark tunnel of disappointment for haunted poor females.
Trial registration number: Applied for registration number. It will be provided at time of presentation.

P-760 Racial differences in birth outcomes in women undergoing ovulation induction (OI)

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Study question: Do reproductive outcomes differ between non-white and white women undergoing OI followed by intrauterine insemination (IUI)?
Summary answer: White women undergoing OI/IUI had a significantly higher clinical pregnancy rate (CPR) and a higher likelihood of achieving clinical pregnancy compared to non-white women.
What is known already: Women of three minority groups in the United States – Black, Asian and Hispanic – have lower birth rates with *in vitro* fertilization (IVF) compared to white women.
Study design, size, duration: This was a retrospective case control using medical records collected in a database of patients who underwent OI/IUI at an academic fertility center. The cohort consisted of 235 white and 166 non-white women between ages 25 and 45 who underwent OI with clomid citrate, letrozole or recombinant FSH followed by IUI between 12/2013 and 4/2014.
Participants/materials, setting, methods: Subjects were matched by age and day 3 FSH and categorized as white or non-white based on self-reporting of race/ethnicity. There was 2:1 matching of white patients to non-white patients. Main outcomes were odds ratios for clinical and biochemical pregnancy as well as CPR and biochemical pregnancy rate (BPR). Data was analyzed using chi-square or Fischer's exact test for categorical variables and *t*-test or Wilcoxon rank-sum for continuous variables.
Main results and the role of chance: Mean duration of infertility for both groups was 21 years. There was a significantly greater prevalence of tubal factor ($n = 6$, 3.6%) and fibroids ($n = 10$, 6.0%) in the non-white group versus the white group ($p < 0.05$). Twenty-five percent of non-white women used donor sperm compared to 12.8% of white women ($p < 0.01$). There were no significant differences between groups in the agent used for OI ($p > 0.05$). CPR was significant higher in white patients ($n = 34$, 14.9%) compared to non-whites ($n = 10$, 6.2%) ($p < 0.05$). BPR was not significantly different (white = 10, 4.4%, non-white = 3, 1.9%, $p > 0.05$). White patients were 2.68 times more likely to achieve clinical pregnancy than non-white patients (CI 95%, 1.28–5.59). White patients were also 2.44 times more likely to achieve biochemical pregnancy compared to non-white patients, but this association was not statistically significant (0.66–9.02).
Limitations, reasons for caution: Due to the retrospective nature of this study, we relied on the accuracy of the electronic record. Furthermore, while this was a case-control and the subjects were matched by age and day 3 FSH, there may have been other confounding factors that we were unable to account for.
Wider implications of the findings: These findings suggest that racial disparities in reproductive outcomes in non-white women are not limited to IVF and extend to OI/IUI cycles. This disparity warrants further investigation into what genetic or biological mechanisms may underlie this inequality.
Trial registration number: Not applicable.

P-761 IVF success rate does not linearly increase with number of collected oocytes when limiting number of fertilised oocytes

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Study question: Does fresh IVF cycle allowing collection of up to 9 oocytes offers similar chances for pregnancy as compared to cycles producing more than 10 oocytes.
Summary answer: In fresh IVF following the fertilisation of up to six MII oocytes. cycles producing 7–9 MII oocytes provided the highest chance for pregnancy
What is known already: Results describing the association between the number of oocytes retrieved and IVF outcomes after fresh embryo transfer are conflicting in the literature. Reasonable limiting of number of fertilised oocytes is claimed to decrease the numbers of frozen embryos while maintaining good overall success rates.

In 2013 Polish government introduced public funding of IVF in which, due to ethical/political reasons the number of fertilised oocytes was limited to six and single embryo transfer was obligatory. In this presentation we review the effect of that in the unselected population of women under 35 years treated by IVF.
Study design, size, duration: The data on 12 011 fresh IVF cycles conducted over a period of 2 years (1st of July 2013 to 1st of July 2015) were collected retrospectively. Stratified analysis was used to evaluate the relationships between the number of retrieved oocytes, quality of embryos and the chances for successful outcome of the treatment.
Participants/materials, setting, methods: Participants: Women younger than 35 years of age treated in IVF programme due to infertility related to endometriosis and male, ovarian, tubal or unknown factors of infertility. Setting: 30 accredited fertility centres in Poland. Methods: retrospective analysis covering clinical data, embryological data and outcomes of fertility treatments.
Main results and the role of chance: The study presents unique clinical material of more than 12 thousand fresh IVF cycles with obligatory limitation of number of fertilised oocytes and obligatory single embryo transfer. In 12 011 fresh IVF cycles conducted on women younger than 35 years of age, oocyte retrieval procedure producing 7–9 oocytes offered best chances for pregnancy following the IVF (2 484 fresh embryo transfers (ETs)/37.04% of clinical pregnancies). When reaching 6 or less oocytes, the clinical pregnancy rates were of 32,19% (of 6 894 fresh ETs). Increased numbers of collected oocytes did not offer improvement of chances for clinical pregnancy – when collecting 10–12 oocytes, 13–15 oocytes and 16 and more oocytes, the clinical pregnancy rates reached 35.09% (1553 ETs); 36.68% (668 ETs) and 34.95% (412 ETs), respectively. Relatively high pregnancy rates might indicate on the need for re-evaluation of current approach of fertilizing all collected and mature oocytes. High numbers of analysed cycles limit the impact of chance in the presented material.
Limitations, reasons for caution: It is a preliminary report covering a period of 24 months of health programme originally scheduled for 3 years, ending in June 2016. Comprehensive analysis will follow after the completion of the programme.
Wider implications of the findings: Study provides data on the impact of limitation of number of fertilised oocytes and requirement for single embryo transfer on success rates of IVF cycles. In spite of apparently limiting factors, the

success rates remained relatively high which brings up the question of optimal number of fertilised oocytes in IVF.
Trial registration number: N/A

P-762 Cultural aspects of oocyte freezing: a pilot survey on knowledge and attitudes in a Swiss female academic population

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Study question: What do young female academics know about fertility preservation through oocyte freezing, what are their opinions and which attitudes do they envisage for themselves?
Summary answer: Most participants knew about oocyte freezing, which was rated more positively if used for medical reasons than for lack of a partner or career choices.
What is known already: In Switzerland, the mean age for women at the birth of their first child rises continually, reaching 31.7 in 2014. Statistics show that 30% of women in their fifties with a university degree are childless and that those with children had less than the number they wished for. Recent studies on fertility awareness and attitudes towards parenthood in academic populations note considerable risks for future involuntary childlessness. The use of oocyte freezing for social reasons is increasing, mostly in the highly educated female population, but generally at ages over 35, which offer lower chances of reaching optimal live birth rates.
Study design, size, duration: This cross sectional exploratory survey aimed to retrieve data from about 100 female academics in a period presumably preceding their wish to start a family, but close enough so that reflecting on childbearing options could be of interest to them. The questionnaire was developed by clinical specialists in ART and research psychologists.
Participants/materials, setting, methods: All female students in a master's class in psychology were invited to fill out the questionnaire in the same sitting, on a voluntary and anonymous basis. Data on previous knowledge of oocyte freezing, as well as the participants' general opinions and personal attitudes towards childbearing, age limits and oocyte freezing for medical or social reasons, were collected. The 95 participants (100% response rate) were aged 21 to 30 (M 23.6; SD 1.98) and were childfree.
Main results and the role of chance: Of the 95 participants, 78.9% knew about the possibility to freeze oocytes. While 9.5% did not wish to have children, the rest indicated the ideal age for themselves as 29 (M = 29.3; SD = 2.4, range 25–39), and the superior limit as 39 (M = 39.0; SD = 3.6, range 31.5–49). The majority (57.9%) noted oocyte freezing as a positive technical advancement, though 2.1% viewed it negatively. While 84.2% saw no religious problem, 34.7% perceived ethical problems such as the high financial cost (69.4%) and the necessity to pose an age limit for childbearing (77.7%). This age limit was noted at 46 years (SD 5.7, range 35–70). Participants were generally favourable towards oocyte freezing in case of chemotherapy (93.7%) or premature ovarian failure (87.4%), but less so if one hadn't found a partner (47.4%), or for career choices (28.4%). Possible personal use of oocyte freezing was rejected by 22.1%, while 77.9% would envisage it for medical reasons, 25.2% for lack of a partner and 9.5% to choose the ideal moment for a pregnancy. Most participants (80%) wished to develop their career before childbearing, 70% had chosen psychology studies in order to balance career and family and for 56.9% oocyte freezing wasn't a good option for attaining this.
Limitations, reasons for caution: The results of this pilot study reflect the attitudes of a sample of highly educated young women, who represent the main population of women using oocyte freezing. Their specialisation in psychology probably indicates heightened interest in socio-cultural issues, and these are perhaps not representative of the general population.
Wider implications of the findings: Young female academics are largely favourable to oocyte freezing for medical reasons, whereas social reasons raise certain ethical questions. Comprehensive information and debating about these issues are important to clarify the influence of socio-cultural factors such as higher education, age and the choice of a partner on future reproductive choices.
Trial registration number: None

P-763 Awareness and attitude of potential Italian oocyte donors towards fertility issues, gamete donation and social freezing: a survey

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Study question: What is the attitude of young Italian women towards fertility issues, gamete donation and social freezing?
Summary answer: Almost half of the responders were not informed or did not have an opinion about fertility issues, gamete donation and social freezing.
What is known already: Gamete donation was routinely performed in Italy until Law 40/2004 on Medically Assisted Reproduction (MAR) prohibited this kind of treatment. In 2014, the Italian Constitutional Court ruled that the ban on gamete donation was unconstitutional, thus restoring the possibility of performing this procedure in those cases where infertility cannot be treated in a different way. At present, gamete donation in Italy is a voluntary act and compensation/reimbursement for donors are not allowed. While finding male donors is quite simple, recruiting female donors appears to be a lot more difficult, also due to the complexity of the procurement procedure.
Study design, size, duration: A survey was elaborated by a working group composed of a MAR specialist, two psychologists, a person working in the field of communication and a statistician. The questionnaire was administered to a sample of 200 women aged between 18 and 34 between December 2014 and February 2015.
Participants/materials, setting, methods: The survey was anonymous and it was composed of 20 questions: 13 were multiple-choice questions, while 7 were open questions. Questions aimed at assessing awareness of responders with reference to issues related to infertility and its treatment and to analyse their attitudes towards these techniques. The survey was administered by a dedicated person at the Bologna University Library.
Main results and the role of chance: The average age was 24.4 ± 4.3. A large majority of responders (90%) declared to know MAR techniques, 10% of them did not. When investigating awareness regarding causes of infertility, the sample split in halves with only 53% of women affirming to know them. A smaller percentage of responders declared to know gamete donation procedures (73.5%). Perception of donors is generally positive, although 42.5% of responders did not know how to answer or are indifferent. This partition of responders will recur also in all questions regarding gamete donation and social freezing. When asked whether they would donate oocytes, the majority of responders (44%) did not know how to answer, while only 19.5% would refuse to do so. In general, those who would be available to donate would also be available to receive gametes. Only 61% of women knew about social freezing and when asked whether they would undergo this procedure, once again a large majority (37.5%) does not know how to answer. Only 44% of patients in favour of social freezing would be available to donate a quota of their oocytes in return for a free freezing procedure.
Limitations, reasons for caution: The results of the survey might be influenced by the size and composition of the specific population analysed. Results might have also been affected by the lack of information on these issues between 2004 and 2014 when the ban on gamete donation was in place.
Wider implications of the findings: Although gamete donation is now legal in Italy, putting it into practice is difficult due to the shortage of donors (especially women) compared to the increasing demand for treatments. The survey shows

that a large share of potential Italian donors appear to be uninterested in or unaware of these issues.
Trial registration number: Not applicable

P-764 Competing risk survival techniques provide accurate estimates of long term outcome in Assisted Reproduction and prognostic factors. A first study in France

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Study question: Estimate the probability and prognostic factors of CLBR, spontaneous birth (SBR) and dropout rates (DOR) for couples undertaking IVF. Appropriate methodology illustrated on French data.
Summary answer: CLBR, SBR and DOR were estimated to 65.4%[60.8, 69.7], 4.2%[2.9–5.3] and 28.1%[24.3–32.3] respectively, all affected by age, number of retrieved oocytes and parity.
What is known already: Cumulative Live Birth Rate (CLBR) probably constitutes the main endpoint of IVF, however its estimation is difficult due to missing values further switch between centers, high variability such as country, time, women age, center effect, and heterogeneity of definitions. Our international meta-analytical calculation provided evidence of a very heterogeneous estimate CLBR of 41.6% [24.3–75.6]. Important difference was suspected among countries, centers, and IVF technique. Substantial number of censored values and need to modeling time to LB necessitates survival techniques. However, CLBR, SBR and DOR constitute three competing risks and cannot be treated by elementary techniques.
Study design, size, duration: We conducted a longitudinal observational national multicenter study on retrospective data of 3 years follow-up, beginning in 2010. Single Stage Cluster Sampling combined with sequential recruitment of patients in each cluster constituted our statistical sampling design. To provide a maximum error of 5% on this sampling plan and assuming intra-class correlation of.08, we planned a representative selection of 40 centers with a balanced recruitment of 100 patients per center.
Participants/materials, setting, methods: In each recruited center, couples starting IVF in the center were sequentially selected, and every cycle was documented. Patients lost to follow-up or switching to other centers were followed by direct contact. CLBR, SBR and DOR were considered as three competing events and studied by a Competing Risk (CR) survival technique. For prognostic models, a multivariate CR technique was based on a backward strategy based on a list of prognostic factors previously reported in literature.
Main results and the role of chance: Out of 58 selected IVF centers, 41 IVF centers accepted to participate, gave full data and recruited 3,806 couples (First Cycle in the studied center Selection). We assessed possible biases further existence of non-participating centers. Our sample was characterized by a median age of 33 years (IQR = 7), mean BMI of 23.4 ± 4.7 and 18% smokers, 74.9% of these couples were childless and a median duration of subfertility of 3 years (IQR = 3). We estimated LB rate to 47.2% at the last news in the centers, 18.9% of cancel due to medical decision or other reasons, and 30.9% lost to follow-up including switching to other centers. These patients were followed by telephone and questionnaire. Patients for which follow up exceeded 3 years were censored. The univariate competing survival model estimated the proportions of CLBR to 65.4% [60.8, 69.7], SBR to 4.2% [2.9–5.3] and DOR to 28.1% [24.3–32.3]. The multivariate Competing survival technique provided evidence of three highly significant ($p < 0.001$) main prognostic factors of LB: women age (OR = 0.94 [0.93, 0.95]), number of previous live birth (OR = 1.32 [1.23, 1.41]), and Number of retrieved oocytes (OR = 1.03 [1.02, 1.03]).
Limitations, reasons for caution: Our estimates and prognosis model remain subject to the center effect which necessitates further analysis. Our first investigation was limited to France, results cannot be generalizable to other countries and require a specific investigation.
Wider implications of the findings: This study based on a standard definition of outcome and use of retrospective data analyzed by competing survival technique provides an accurate frame to investigate and compare long term outcome with other countries. Results highlight that CLBR observed in France is similar to CLBR calculated from the international meta-analysis.
Trial registration number: not applicable

P-765 Lead levels and IVF outcome: the case of "Land of fires" (Campania, South Italy)

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Study question: To evaluate the correlation between lead levels in biological fluids and fertility in women undergoing *in vitro* fertilization procedures (IVF) residents in the "Land of fires".
Summary answer: High intrafollicular lead levels are correlated to reduced ovarian reserve and lower pregnancy rate in the land of fire.
What is known already: Three decades of illegal practices of waste dumping and consequent environmental abuse have made specific areas of Campania (Southern Italy), currently defined as "land of fires", a unique case in the context of waste-related health outcomes. Scientific evidence is mounting in support of a significant increase in cancer mortality and malformation occurrence, but reproductive health has never been investigated. To date, literature provide few data on the possible relationships between heavy metal exhibition and female reproductive potential. Some evidence indicates a higher incidence of infertility and miscarriages among women occupationally exposed to heavy metals.
Study design, size, duration: In this prospective study 99 women undergoing IVF cycles were enrolled from September 2014 to August 2015. The study group (A Group) included 53 women resident in "land of fires"; the control group (B group) included 46 women resident far from polluted areas. Lead level were evaluated in biological fluids.
Participants/materials, setting, methods: Exclusion criteria: lead occupational exposure; smoke and alcohol abuse. Urine, blood and follicular fluid samples were collected. Lead levels were determined by atomic absorption spectrometry with graphite furnace in dual beam. Ovarian reserve was defined by antral follicular count (AFC) and AMH determination. Pregnancy rates were evaluated as indicator of fertility health. Data were analyzed using the unpaired Student's *t*-test (p-value ≤ 0.05).
Main results and the role of chance: Patients living in areas included in the "Land of fires" have intrafollicular lead levels higher compared to the control group, 1.78 ± 1.45 μg/L and 0.73 ± 0.57 μg/L respectively ($p \leq 0.05$). Not statistically significant differences were found in blood lead levels and piomburia between two groups. The pregnancy rate is 17% in A group and 28% in B group, reaching a statistical significance ($p \leq 0.05$). Among A group patients, pregnant patients showed lower intrafollicular lead level compared with non pregnant patients ($p \leq 0.05$). High intrafollicular lead levels were associated with reduced ovarian reserve. The discrepancy between lead intrafollicular concentrations and blood and urine concentration may underlie a mechanism of intraovarian accumulation of lead due to impaired clearance or a high follicular membrane permeability. The correlation between the higher intrafollicular leads concentrations, reduced ovarian reserve and lower pregnancy rate observed in the study group lead to hypothesize that chronic exposure to lead and its transit through the follicular membrane may results in an intraovarian toxic accumulation of the metal. In this contest lead could act as endocrine disruptor for direct damage on the granulosa cells. The result is reduced ovarian reserve and impaired fertility.
Limitations, reasons for caution: Small sample size. Environmental lead concentration not evaluated.

Wider implications **of the findings:** This is the first research that evaluates lead effects on IVF outcome in the "land of fires". Impaired fertility in the "Land of fires" confirm the need to start investigations for the assessment of individual exposure and the resulting reproductive risk thought an analysis of the environmental contamination.
Trial registration number: Not requested

P-766 The Polish state-funded program – infertility treatment with *in vitro* fertilization for years 2013–2016. results after the first 2 years

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Study question: What are the preliminary results of the Polish state-funded programme for infertility treatment with *in vitro* fertilization (IVF) achieved between 2013 and 2015?
Summary answer: Despite significant limitations in the number of fertilized eggs (only six) the results are generally in agreement with data obtained from European registers by ESHRE.
What is known already: It is for the first time that Polish government introduced public funding of *In Vitro* Fertilization (IVF). The programme is exceptional in a way that due to ethical/political reasons the number of fertilized oocytes was limited to six and single embryo transfer was obligatory. In this presentation we summarize the preliminary results of the first 2 years of the programme which will end in June 2016.
Study design, size, duration: The IVF programme approved 3 IVF procedures for women under 40 years of age. For women under 35 years the number of fertilised eggs was limited to 6 and implemented obligatory SET (single embryo transfer). For women over 35 years there were no limitations on the number of eggs fertilised and DET (double embryo transfer) was approved. The presented results were achieved between 2013 and 2015.
Participants/materials, setting, methods: Participants: A total of 19 345 initiated ovarian stimulation cycles in various protocols have been registered in the 2 years period. Setting: The IVF program was conducted in 31 clinics subjected to inspections and required the clinics to report results within 2 weeks of finalizing a procedure. Methods: retrospective analysis covering clinical data, embryological data and outcomes of fertility treatments.
Main results and the role of chance: Clinical pregnancy rates per cycle and embryo transfer were 29.9% and 32.9%, respectively. In FET (Frozen Embryo Transfer) cycles, the pregnancy rate per thawing was 28.32% and per SET and DET were 26.7% and 37.5%, respectively. Treatment cycles reported from all over the country resulted in the cumulative pregnancy rate per cycle of 41.37%. Clinical pregnancy rate per fresh embryo transfer in the group of women under 35 years was 33.9% and multiple pregnancy rate was 4.8%. In the group of women between 35 and 40 years the pregnancy rate per fresh embryo transfer was 30.9% and multiple pregnancy rate was 12.6%. Miscarriage rate was 15.22% and single pregnancy rate was 95.2%. OHSS (Ovarian Hyperstimulation Syndrome) rate was 2.07%. High numbers of analyzed cycles limit the impact of chance in the presented material.
Limitations, reasons for caution: This is only a preliminary data obtained after two initial years. The results will be complete when all available live-birth data can be analysed.
Wider implications of the findings: The success rates of the programme remained relatively high, despite limiting factors, which may have a significant implications for future plans of public-funded IVF programmes.
Trial registration number: N/A

P-767 Effects of laboratory procedures on intrauterine insemination success

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Study question: What is known about the laboratory procedures of IUI, including the pre- and post-analytical conditions, and how do they relate to the pregnancy rates?
Summary answer: Few laboratory procedures were associated with the pregnancy rates of IUI, but most other procedures were characterized as inconclusive.
What is known already: Several variables were reported to influence the outcome of IUI. Most of these variables are related to patient characteristics or stimulation protocols. The study of the association between laboratory procedures and pregnancy rates, however, is neglected. Despite the protocol described in the laboratory manual of the World Health Organization (WHO, 2010), most laboratories still use their own procedures. The aim of this study is to identify the association of the laboratory procedures and the gaps of knowledge, which could be used for the development of international clinical guidelines.
Study design, size, duration: A systematic review focusing on the influences of semen collection (i.e., ejaculatory abstinence, ejaculatory frequency, collection place), semen processing (i.e., separation method, temperature during centrifugation/storage), insemination (i.e., timing of IUI, bed rest after IUI) and the used devices on pregnancy outcomes. The literature search was based on specific key words in different databases. The levels of evidence of the main conclusions are reported, according to the National Institute for Health and Clinical Excellence (NICE, 2013).
Participants/materials, setting, methods: The literature search was performed in Medline and the Cochrane library. The references and related citations of suitable studies were reviewed as well. When there were no studies studying the association between the laboratory procedures and the pregnancy rates of IUI, we reviewed the association with sperm parameters. When sufficient literature was available, we based our conclusion on the studies with the highest level of evidence according to the NICE guideline.
Main results and the role of chance: The review of literature is for most variables characterized by a low level of evidence, a limited number of studies or an inadequate outcome measure (i.e., sperm parameters instead of pregnancy rates). The comparison of several laboratory procedures revealed that they did not influence the IUI outcome. The pregnancy rates were not related to the different options of the place where the semen sample was collected, the temperature during centrifugation, the method used for the timing of IUI, the time interval between ovulation and IUI, and the insemination catheter. A positive correlation was found between the pregnancy rates of IUI and an ejaculatory abstinence period of up to 2–3 days and bed rest of 10–15 min after insemination (compared to longer periods of abstinence and direct mobilization, respectively), although a limited number of studies was performed on these subjects. The reported influences of the different separation techniques and the time intervals between semen collection, semen processing and IUI revealed no consensus in study results, leading to the inability to select a superior method or time interval.
Limitations, reasons for caution: The review of studies on reproductive medicine often reveals a lack of standardization in inclusion criteria and methodologies in these studies. Study results can differ among these determinants, which makes it sometimes impossible to draw strict conclusions based on previous studies.
Wider implications of the findings: Based on the literature, it is impossible to develop an optimal IUI treatment protocol. This review emphasizes, once again, the need for standardization in assisted reproductive studies technologies. In this way, it will be possible to identify -and to bridge the knowledge gap, in order to improve the protocol.
Trial registration number: Not applicable

P-768 Preconceptual health and fertility treatment – an evidence based approach

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Study question: What is the existing evidence around preconceptual health and reproductive outcomes for couples who are seeking fertility treatment?
Summary answer: Positive lifestyle modification and the optimization of the preconceptual period for both men and women can influence the outcome of assisted reproductive technology.
What is known already: A large percentage of couples attending a fertility clinic face multiple lifestyle issues but do not always receive appropriate preconcepual advice (Anderson et al., 2010). There is accumulating evidence demonstrating that positive lifestyle modification and the optimization of the preconceptual period for both men and women can influence the couple's reproductive potential (Clark et al., 1995; Klonoff-Cohen, 2005; Dondorp et al., 2010; Sharma et al., 2013).
Study design, size, duration: A thorough electronic search was conducted in PubMed, Embase, Medline and Cohrane databases and references of relevant studies were cross-checked in order to include all relevant human studies from 1946 until December 2015 linking preconceptual behaviors with the outcome of fertility treatment. The computerized search explored the importance of numerous factors such as smoking, caffeine and alcohol consumption, obesity, physical exercise, stress, recreational drug use, diet (including vitamins, micronutrients, antioxidants, minerals), alternative medicine, environmental factors and pollutants.
Participants/materials, setting, methods: The Mesh terms included caffeine (coffee, tea, soft drinks), smoking, BMI/obesity, alcohol, recreational drugs (cocaine, cannabis, opioids, methadone, heroine, anabolic steroids), exercise, diet (Vitamins A, C, D, E, multivitamin supplements, selenium, zinc, coenzyme q10, L arginine, iron, copper, antioxidants, folate, melatonin, carnitines, isoflavones), genetic conditions, acupuncture, environment (perfluorinated chemicals. pesticides, herbicides, mercury, lead, solvents, cell phone, laptop, phthalates, phenols, cosmetics), stress "AND" fertility/IVF (*in vitro* fertilization) for men and women.
Main results and the role of chance: A total of 398 relevant studies were identified. Negative parental lifestyle behaviors can affect the outcome of fertility treatment and could even have transgenerational effect on the fertility of the offspring as demonstrated by human and animal studies (Lutterodt et al., 2009; Anderson et al., 2014; Fowler et al., 2014; Sobinoff et al., 2014). The evidence is stronger for some of the factors examined such as smoking, physical exercise, balanced diet and body mass index whilst more research is needed around other factors such as a variety of diet supplements, environmental exposures, stress reducing techniques and even caffeine intake before they are incorporated in the counseling for subfertile couples.
Limitations, reasons for caution: Large epidemiological studies and randomized controlled trials are needed for solid conclusions to be drawn in order to offer clear advice to infertile couples as a big part of the existing evidence is retrospective in nature.
Wider implications of the findings: Proper preconceptual care and counseling for couples undergoing fertility treatment can improve success rates and also prevent pregnancy complications. Adequate preconceptual counseling incorporating this evidence is essential.
Trial registration number: Not applicable

P-769 Are non-phthalate substitute plasticizers safe? The association of urinary di-isononyl cyclohexane-1,2-dicarboxylate (DINCH) metabolites with *in vitro* fertilization (IVF) outcomes: results from the EARTH study

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Study question: Are concentrations of urinary DINCH (Ur-DINCH) metabolites [MHiNCH and MCOCH, cyclohexane-1,2-dicarboxylic acid-mono(hydroxy-isononyl) ester and cyclohexane-1,2-dicarboxylic acid-mono(carboxy-isooctyl) ester, respectively] associated with IVF outcomes among women attending an academic fertility center?
Summary answer: There was a suggestion of an inverse association between urinary MHiNCH (Ur-MHiNCH) concentrations and number of retrieved and mature oocytes.
What is known already: Over the last decade, non-phthalate plasticizers (such as DINCH) are increasingly used as "safer" alternatives to ortho-phthalate esters in food packaging and in the manufacturing of toys, building materials and medical devices. Since 2002, DINCH metabolites have been increasingly detected in urine samples collected from children and adults in both Europe and the US. Due to its increased use in the manufacture of various products, higher and more widespread human exposure to DINCH is expected in the future. To our knowledge, there are no data on the potential human health effects of DINCH.
Study design, size, duration: Prospective cohort of 118 women undergoing 160 fresh IVF cycles at the Massachusetts General Hospital in Boston, MA, USA (06/2011–06/2015). All women participated in the Environment and Reproductive Health (EARTH) study and provided up to two urine samples prior to oocyte retrieval (285 urine samples).
Participants/materials, setting, methods: We quantified the urine specific-gravity adjusted concentrations of Ur-DINCH metabolites (MHiNCH and MCOCH, Ur-MHiNCH and Ur-MCOCH, respectively) by isotope dilution tandem mass spectrometry. Intermediate and clinical endpoints of IVF treatments were abstracted from electronic medical records. We used generalized linear mixed models to evaluate the association between Ur-DINCH metabolites concentrations and IVF outcomes, with random effects to account for multiple IVF cycles, and adjusting for age, year of treatment and infertility diagnosis.
Main results and the role of chance: For MHiNCH and MCOCH, 49.3% and 32.3% were above the limit of detection, and urine specific-gravity adjusted concentrations ranged up to 42.8 and 16.0 µg/L, respectively. For statistical analysis we defined three groups for MHiNCH (low/medium/high) and two groups for MCOCH (low/high). The groups did not differ significantly by participant baseline characteristics. Increased specific-gravity adjusted Ur-MHiNCH concentrations were associated with a decreased yield of retrieved and mature oocytes, with adjusted means (95% CI) for low, medium and high Ur-MHiNCH concentrations of 13.0 (11.7–14.6), 12.2 (10.6–14.2), and 10.9 (9.2–13.0)] for number of retrieved oocytes; and 10.5 (9.4–11.8), 9.7 (8.3–11.3), and 9.0 (7.6–10.7), for number of mature oocytes, respectively. Women in the highest group of specific-gravity adjusted Ur-MHiNCH concentrations had a 16.2% and 14.3% decrease in the number of retrieved and mature oocytes, respectively, compared with women in the lower group (p-trend = 0.08 and p-trend = 0.10, respectively). Specific-gravity adjusted Ur-MHiNCH concentrations were not associated with other IVF outcomes (proportion of best embryos, p-trend: 0.66; rates of fertilization, p-trend: 0.61; implantation, p-trend: 0.96; clinical pregnancy, p-trend: 0.77; and live-birth, p-trend: 0.49). For MCOCH, there were no associations between specific-gravity adjusted urinary concentrations and any of the IVF outcomes measured.
Limitations, reasons for caution: DINCH metabolites were measured in urine samples collected during the IVF cycle and reflect only short-term exposure. Measured urinary concentrations might not accurately represent long-term exposure and its contribution to the observed early IVF outcomes. Results may not be generalizable to women conceiving naturally.
Wider implications of the findings: Higher Ur-MHiNCH concentrations were associated with lower yield of retrieved and mature oocytes among women undergoing IVF, suggesting that exposure to certain non-phthalate plasticizers might affect fertility. However, further studies are required, since this is the first human study evaluating potential adverse effects of non-phthalate plasticizers on reproductive health.
Trial registration number: None.

The above findings/conclusions are those of the authors and do not necessarily represent the official position of the Centers for Disease Control and Prevention (CDC).

P-770 Population based Northern Finland Birth Cohort 1966 study shows serum lipids and metabolic syndrome to have association with an increased risk of uterine fibroids

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Study question: Are uterine fibroids associated with increased cardiovascular risk?
Summary answer: We herein report of an association between increased serum lipids and metabolic syndrome with increased risk of uterine fibroids.
What is known already: Uterine fibroids are the most common tumour in females. Recent studies suggest similarities in biological disease mechanisms for fibroids and atherosclerosis. Similar risk factors have been associated with both conditions: obesity, hypertension, and abnormal serum lipids. These findings are awaiting confirmation that a population based follow-up study could offer with extensive health examination data collection linked with a national hospital discharge register.
Study design, size, duration: The Northern Finland Birth Cohort (NFBC1966) is a population-based prospective study including all children born in 1966 in the Northern Finland area. The data were collected from national registries, postal questionnaires and clinical health examinations. All females included in the NFBC 1966 who underwent an extensive clinical health examination at age 46 years (n = 3,268) comprised the study population for this study.
Participants/materials, setting, methods: All females included in the NFBC1966 who were alive and traceable (n = 5,118) were invited for the 46-year follow-up study; 3,268 (63.9%) responded, returned the postal questionnaire and attended the clinical examination. Uterine fibroid cases were identified through the national hospital discharge register that has data on disease diagnoses based on WHO ICD-codes. Uterine fibroid codes, ICD-9: 218 and ICD-10: D25 were used for case identification. Self-reported fibroid cases were identified through the postal questionnaire.
Main results and the role of chance: A total of 729 fibroid cases were identified through WHO ICD disease codes for fibroids and self-report. With logistic regression analysis, the odds ratio of risk of uterine fibroids (all cases, and ICD code identified cases) according to serum lipid profile, metabolic syndrome by the International Diabetes Federation (IDF) definition and body composition was estimated. With adjustment for parity, education and BMI, the risk of fibroids rose significantly for every 1 mmol/l increase in LDL and triglycerides, (OR = 1.14 95% CI 1.02, 1.26 vs. OR = 1.27 95% CI 1.09, 1.48). The risks were higher for hospital discharge-defined (ICD code based) fibroid diagnosis (OR = 1.22 95% CI 1.05, 1.42 vs. 1.37 95% CI 1.10, 1.68). With the same adjustment model, IDF-defined metabolic syndrome raised the risk of hospital discharge-based fibroid diagnosis significantly (OR = 1.50 95% CI 1.10, 2.03). Additionally every 1 cm increase in waist circumference increased the risk of fibroids (all fibroids: OR = 1.02 95% CI 1.00, 1.04; hospital discharge-based fibroid diagnosis: OR = 1.03 95% CI 1.01, 1.06).
Limitations, reasons for caution: The majority of fibroid cases were identified by self-report only. There was likely an under-identification of cases and misclassification of some cases as controls; this would have diluted the effects of reported associations. The data analysed were cross-sectional and therefore cause and effect for the associations observed cannot be distinguished.
Wider implications of the findings: Increased serum lipids and metabolic syndrome are associated with increased risk of uterine fibroids. Along with central obesity these findings add to an increased risk for cardiovascular disease among women with fibroids. These observations might also suggest that metabolic factors could have roles in underlying biological mechanism in fibroid development.
Trial registration number: *

P-771 Oocyte donation in India – characterisation of donors

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Study question: The primary objective of the study was to identify the factors which motivate women to donate oocytes.
Summary answer: All donors were from a low socio-economic background and the primary motivation for oocyte donation was financial gain.
What is known already: Advancements in assisted reproductive technology in the form of oocyte donation has given hope to achieving parenthood in women who do not have healthy oocytes. There are two main kinds of oocyte donors – family/friends and anonymous (generally referred to as altruistic donors). In India, all donors are anonymous as family/friends are not permitted.
Study design, size, duration: A cross-sectional survey was administered to 20 consecutive women who registered for anonymous oocyte donation over a 6-month period from July 2015 to December 2015 at a specialised tertiary infertility clinic in North India.
Participants/materials, setting, methods: A professionally trained psychologist with experience in counseling, administered the questionnaire to the oocyte donors after taking their consent. The questionnaire had two main domains – (1) basic demographic and socio-economic information (2) factors motivating for oocyte donation.
Main results and the role of chance: The age range of the 20 respondents was 21–28 years. 20% had had no formal schooling; 30% had attended primary school and the remaining 50% had attended/completed secondary school. No respondent had done graduation or post-graduation. A third of the respondents were not formally employed and the remaining two thirds were mainly employed as unskilled workers. The primary factor motivating the respondents was financial gain (95%) and altruism (5%). However, 60% of the respondents did derive satisfaction at being able to assist couples desiring parenthood. 40% of respondents would consider repeat donation for further financial benefit. Importantly, prior to the counselling, 20% of respondents had not completely understood the implications of being an oocyte donor.
Limitations, reasons for caution: Small sample size and use of a questionnaire. Further exploration by qualitative interview techniques assessing the motivation to donate oocytes as well as the impact following oocyte donation on the physical and psychological well-being of the donor will be helpful.
Wider implications of the findings: The knowledge that the primary motivating factor for individuals to donate oocytes is financial gain, would necessitate development of policy which safeguards the interest of the donors as well as the recipients. Such a policy is currently lacking in India.
Trial registration number: Not applicable

P-772 Impact of geographical origin on IVF results: a monocentric French retrospective observational cohort study evaluating three populations originating from Europe and North or Sub-Saharan Africa

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Study question: Is geographical origin an independent factor impacting ovarian response to IVF stimulation and IVF outcomes?
Summary answer: After adjusting for confounding factors, no difference was found in IVF outcomes according to geographical origin, except for the estradiol peak, higher in sub-Saharan women.
What is known already: First American studies described an association between ethnicity and poorer IVF outcomes in African-American and Asian minorities. In these studies, socio-economic, as well as environmental and genetic factors were incriminated. Despite the recent migration context, different from the situation of African-Americans, a recent UK study showed that African or Asian ethnicity was an independent risk factor for worse IVF outcomes, even after adjusting for several biases. Ovarian response to stimulation was less studied than IVF outcomes. Differences were sometimes noticed, in particular a higher estradiol (E2) level on the day of hCG triggering and a increased number of oocytes retrieved.
Study design, size, duration: A monocentric French retrospective observational cohort study in a public academic infertility unit characterized by a wide variety of patient's geographical origins was conducted. The three main groups were almost equally originating from Europe, Sub-Saharan Africa (SSA) and North-Africa (NA)(Maghreb). Data from January 2013 to June 2015 were analysed involving a total of 812 first IVF cycles. Main outcome measures were stimulation data and clinical pregnancy rates per oocytes retrieval and embryo transfer.
Participants/materials, setting, methods: Data for all women undergoing their first IVF cycle during the study period were analysed for ovarian stimulation and IVF outcomes according to their geographical origin. Data were retrospectively collected in the global IVF attempts database: *IVF Medifirst*

software. Stimulation protocol choice, oocyte retrieval timing and fertilization technique were based on identical criteria for the three groups. General linear models including ANOVA and multiple regressions were used for statistical comparison and adjustments.
Main results and the role of chance: Eight hundred and twelve women were included: 286 from Europe, 292 from SSA and 234 from NA. All women were comparable for age and ovarian reserve parameters. SSA and NA women had higher BMIs, were less nulliparous, less often smokers, and received more agonist protocols than Europeans. SSA women were more likely to be infected by HIV or HBV than Europeans (22.9% vs. 2.8% for HIV). Duration of stimulation, number of oocytes retrieved, mature oocytes and embryos were similar in the three groups but total dose of gonadotropins, peak of E2 and cancellation rate were increased in SSA women compared to Europeans. After adjusting for age, BMI, primary infertility, protocol, smoking status, viral infection and fertilization technique, only the peak of E2 (1,698 ± 867 pg/ml vs. 1,454 ± 681) and the cancellation rate (28.8% vs. 19.6%) remained significantly higher in SSA women. Compared to Europeans, without adjustment, SSA women had poorer IVF outcomes despite the same number of embryo transferred (clinical pregnancy rate per transfer 20.1% vs. 31.4%). After adjustment, this difference did not reach significance. Nevertheless, after adjusting for the same parameters except HIV, pregnancy rates were significantly poorer in SSA women. Concerning NA women, no difference with Europeans was noticed in IVF stimulation or results.
Limitations, reasons for caution: Although the study size was important, adjusting for many factors to avoid confounding bias decreased the strength of the study. It generated no difference in IVF results without any lesson to be drawn in daily practice. A case-control study, considering ethical issues, could be a better approach for future studies.
Wider implications of the findings: Many confounders can conceal the independent impact of geographical origin. Based on our results, no specific adaptation of gonadotropin dose or embryo transfer policy depending on geographical origin seems needed. Only the usual criteria should be used such as age, BMI or HIV infection status for our everyday clinical practice.
Trial registration number: N/A

POSTER VIEWING SESSION

REPRODUCTIVE SURGERY

P-773 Hysteroscopic septum resection versus expectant management for women with a septate uterus

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Study question: Does hysteroscopic septum resection improve reproductive outcome in women with a septate uterus?
Summary answer: Septum resection is not superior to expectant management in improving reproductive outcome in women with a septate uterus, but good quality data is missing.
What is known already: Women with a septate uterus are at increased risk for miscarriage, subfertility and preterm birth. Resection of the septum could improve reproductive outcome in these women and is considered to be the standard of care in many countries. However, data on the effectiveness of this surgical procedure are scarce.
Study design, size, duration: We systematically reviewed the literature for studies that assessed the effect of hysteroscopic septum resection in women with a septate uterus.
Participants/materials, setting, methods: The Cochrane Menstrual Disorders and Subfertility Group searched their specialized Register, the Cochrane Central Register of Controlled Trials (CENTRAL), MEDLINE, EMBASE and PSYCHINFO (inception to September 2015), one reviewer searched other registers for unpublished dissertations and theses. In this search, only randomised controlled trials (RCTs) were eligible for inclusion. In addition we searched the abovementioned databases for retrospective and prospective comparative studies.
Main results and the role of chance: We did not identify randomised controlled trials on the topic. There were nine comparative non-randomised studies, three of which were prospective and six were retrospective studies. Three studies included women with recurrent miscarriage, three studies women with subfertility and three studies included both. The chances of a live birth were significantly lower in the septum resection group than in the expectant management group (7 comparative studies, n = 338 vs. n = 296, OR 0.4, 95% CI 0.2–0.6, I^2 for heterogeneity = 18%). Clinical pregnancy rates were not different (9 comparative studies, n = 518 vs. n = 451, OR 1.6, 95% CI 0.5–5.4), I^2 for heterogeneity = 75%).
Limitations, reasons for caution**:** There was a high risk of bias as none of the studies was randomised, all but one study were single-centre studies and only 3 studies were prospective studies. Also, heterogeneity was considerable, especially for clinical pregnancy.
Wider implications of the findings: This systematic review shows there is no sound evidence that septum resection is superior to expectant management in women with a septate uterus. This procedure should not be performed, unless as part of a randomised controlled trial. Such a trial is urgently needed, and currently halfway (TRUST study, NTR 1676).
Trial registration number: Not applicable

P-774 Proinflammatory cytokines TNF-α and IL-1 in pelvic peritoneal adhesions of various etiology at reproductive age women

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Study question: To study the expression of TNF-α and IL-1 in the tissue of the pelvic peritoneal adhesions of various origins in women of reproductive age.
Summary answer: Morphological examination and immunohistochemical study of the pelvic peritoneal adhesions revealed differences in the expression of cytokines IL-1 and TNF-α depending on the genesis.
What is known already: One of the reason identifies the high frequency of adhesion formation is the presence of inflammation in the abdominal cavity with different severity and origin. Activation of intercellular relationships in the peritoneum becomes promoter of further adhesions when inflammation. It is known that TNF-α and IL-1 are among the most important pro-inflammatory cytokine that effect cells and tissues both by means of paracrine and endocrine mechanism. Data on expression of TNF-α and IL-1 in the pelvic peritoneal adhesions in connection with their prescription, localization and origin is absent at accessible literature.
Study design, size, duration: One hundred infertile women (aged 19–49 years) with pelvic peritoneal adhesions, who were underwent operative laparoscopy. 38 patients with a history of chronic inflammatory diseases of pelvic organs; 32 patients with endometrial disease (12 patients with endometrioma and 20 patients with external peritoneal endometriosis) and 30 patients who had undergone previous surgery for pelvic and abdominal cavity took part in this study.
Participants/materials, setting, methods: The material for this study was the fragments of surgical material (adhesions and their parts) n = 100, taken from the women of reproductive age who suffered with infertility during operative laparoscopy. The morphological and immunohistochemical study of adhesions were carried out by standard techniques using paraffin blocks, reagents of Dako and monoclonal antibodies to TNF-α (Anti-TNF alpha antibody (ab6671)) and IL-1 (Anti-IL-1alpha antibody [as5] (ab17281)) of Abcam with automatic coloring Dako Cytomation.
Main results and the role of chance: Immunohistochemical study of tissue adhesions obtained from the women who underwent surgeries on the pelvic organs, the expression of TNF-α was extremely low and was equal to 19 ± 0.3 points. Only a few positively stained macrophages were found in a limited number of observations. Also in this material IL-1 was characterized by an extremely low level of expression and was equal to 13 ± 0.4 points, in some observations there was no expression of this marker. During the immunohistochemical study

of adhesions in women with a history of inflammatory diseases of the pelvic organs, there was a mild TNF-α, i.e., it was 35 ± 0.2 points. Positive staining was detected mainly in the mesotheliocytes' cytoplasm covering the adhesions, at least in lymphoid-macrophage clusters. IL-1 is also detected in meager quantities, mainly in the cytoplasm of mesothelial cells was 17 ± 0.1 points. Immunohistochemical study of the material obtained adhesions in patients with external genital endometriosis carried out in the first phase of the cycle was characterized by a moderate expression of TNF-α and IL-1 in the mesothelial cells, lymphocytic-macrophage aggregates and endometriosis. Expression of TNF-α was 151 ± 0.5 points and IL-1 – 148 ± 0.2 points.
Limitations, reasons for caution: Age limitation, only women aged 19–49 yrs took part in this study. Exclusion criteria were the following for the groups: acute gynecological diseases, malignant diseases of female genitalia and ovarian tumors.
Wider implications of the findings: The extremely low level of TNF-α and IL-1 expression was found in postoperative and inflammatory adhesions. The highest degree of these cytokines was detected in patients with external genital endometriosis. Thus, in case of endometriosis, the adhesion's tissue by itself is the site of the induction of inflammation in the pelvis.
Trial registration number: Case control study.

P-775 The experience of absorbable knotless wound closure device used in laparoscopic myomectomy

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Study question: The laparoscopic surgery has more several advantages than traditional explore laparotomy. Comparison with handiwork, operating by slender instrument is more imprecision to sew and tie.
Summary answer: Absorbable Knotless Wound Closure Device is a device with the efficient barb and welded loop design. It help surgeon close wound stabilized and tie rapidly.
What is known already: Many surgeries are accomplished by virtue of it nowadays. Hemostatic handing, suturing, and renovating of leiomyoma are the most important procedures and they will be operated by slender instruments with stitch suture and tie. Comparison with handiwork, operating by slender instrument is more imprecision to sew and tie. So that it may bring on following situations such as joints between tissue not rigid enough or difficulties for stypsis on suture and cross-cut. We apply the device in laparoscopic myomectomy and hope to use it can diminish surgery time, blood loss, complication but better to uterus recovery.
Study design, size, duration:
Materials and methods: this prospective study evaluated data on the efficacy of treatment in the 62 women who underwent laparoscopic myomectomy at Taipei City Hospital Zhongxiao Branch from January 2010 through August 2012. The patients were randomly assigned to Absorbable Knotless Wound Closure Devicegroup and 2–0 vicryl suture group.
Participants/materials, setting, methods: We applied this device to laparoscopic myomectomy with the aim of diminishing surgery time, reducing blood loss and reducing the number of complication; such improvement ought to result in better uterus recovery.
Main results and the role of chance:
Result: The two groups were similar in terms of age, body weight, delivery number, hemoglobin and hematocrit before surgery. After surgery, blood loss, operation time, change in hemoglobin, change in hematocrit and the presence of uterus defects among the Absorbable Knotless Wound Closure Device group were significant less than among the vicryl group. There are 26 patients pregnant after accepted surgery within 2 years. 12 of them are classified as Absorbable Knotless Wound Closure Device and the others are 2-O Vicryl. Among these 26 pregnant patients, 1 patient classified as 2-O Vicryl has received Hysterectomy cause of uterine rupture on the timing of the 24 week during her pregnancy, and the others have taken Cesarean Section successfully for childbirth.
Limitations, reasons for caution: This is one hospital experience and less cases (62 cases) study. The more experience were need.
Wider implications of the findings:
Conclusion: The Absorbable Knotless Wound Closure Device allows the surgeon to stably close any wound rapidly without having to tie-off separately. The wound margin is held firmly together. In conclusion, the Absorbable Knotless Wound Closure Device brings a lot of benefits when used for laparoscopic myomectomy.
Trial registration number: TCHIRB-10402105-E

P-776 Risk factors for parasitic myoma after use of morcellator in laparoscopic myomectomy: multicenter experience and literature review

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Study question: What are the risk factors involved in the appearance of the parasitic myoma after laparoscopic myomectomy using morcellation?
Summary answer: The type of morcellator used in laparoscopic myomectomy is an important risk factor in the appearance of the parasitic myoma.
What is known already: The most important risk factor involved in the appearance of the parasitic myoma described in the studies published until present in the literature is leaving fragments of the uterus or myoma tissue in the abdominal cavity after morcellation.
Study design, size, duration: Multicenter retrospective study realized on 720 cases of laparoscopic myomectomy divided in two groups. The first group of patients were operated between 2006 and 2010 and we used Sawalhe super cut II Karl Storz for morcellation, while the second group of patients was operated during 2010–2016 and we used RotoCut G1 Karl Storz.
Participants/materials, setting, methods: From the first lot of patients (320 cases) we diagnosed 2 cases of abdominal polifibromatosis at 13, respectively 18 months postmyomectomy. The diagnosis was established clinically and imagistically (ultrasound, MRI) in both cases. The myoma nodules were excised by laparotomy (we excised totally 6, respectively 12 nodules).
Main results and the role of chance:
Limitations, reasons for caution: Using a right sheath morcellator increases the risk of excessive fragmentation of the myoma during morcellation. The morcellators with a 45 degrees angle sheath favor the peeling effect, decreasing thus the risk of appearance of small fragments which can disseminate in the abdominal cavity.
Wider implications of the findings: Using a right sheath morcellator increases the risk of excessive fragmentation of the myoma during morcellation. The morcellators with a 45 degrees angle sheath favor the peeling effect, decreasing thus the risk of appearance of small fragments which can disseminate in the abdominal cavity.
Trial registration number:

P-777 Copper intrauterine device improves pregnancy outcome in women with repeated implantation failure

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Study question: Does clinical pregnancy rate increase in sterility women with repeated implantation failure by placing copper intrauterine device (Cu-IUD)?
Summary answer: Cu-IUD effectively improve clinical pregnancy rate for patients with repeated implantation failures.
What is known already: Repeated implantation failure (RIF) is still a problem for many patients and their physicians. Some interventions have been practiced to overcome this problem, including utilization of adjuvant to enhance oocyte number and quality, endometrial injury to improve poor endometrial response, assisted hatching and preimplantation genetic screening to correct embryonic factors, as well as Chinese herbal medicine and acupuncture. Although previous studies have shown that hysteroscopy improved pregnancy outcomes in women

with or without intrauterine abnormalities, the effect of hysteroscopy and Cu-IUD on pregnancy rate is unclear.

Study design, size, duration: A retrospective cohort study was conducted involving 440 sterility women with RIF at our center during 2014. RIF is considered to exist in women who do not achieve clinical pregnancy after two or more transfers with at least one good-quality embryo. Of the 440 patients, 382 were in Cu-IUD group, and 58 were in non-IUD group. The pregnancy outcomes were followed up to 6 months after hysteroscopy.

Participants/materials, setting, methods: Women with RIF aged 18 to 45 were recruited. Patients with congenital uterine malformation were excluded. All patients were examined with hysteroscopy. If endometrial polyps, or slight intrauterine adhesions were found, pruning was conducted. Cu-IUD was inserted immediately after hysteroscopy in Cu-IUD group and removed after two menstrual periods. For non-IUD group, embryo was implanted the next month after hysteroscopy. Clinical pregnancy and Implantation data were collected up to 6 months after hysteroscopy.

Main results and the role of chance: Patients'characteristics such as age, BMI, infertility duration, cause of infertility were comparable among the two groups. The most common abnormalities in women with RIF included endometrial polyps or polypoid endometrium, chronic endometritis and intrauterine adhesions. Clinical pregnancy rates in Cu-IUD group were significantly higher than in the non-IUD group (43.96% vs. 33.56%, respectively, $P < 0.05$). Following the office hysteroscopy and Cu-IUD administration, a significant increase was observed in the implantation rate of women with RIF (28.96% vs. 20.37%, respectively, $P < 0.05$) compared to non-IUD group. There were no significant differences in early abortion, ectopic pregnancy, and late abortion rate between Cu-IUD group and non-IUD group. Removal of endometrial polyps, polypoid endometrium, and slight intrauterine adhesions with hysteroscopy improves the pregnancy rate. In addition, the mechanical endometrial injury caused by hysteroscopy may have a positive prognostic value for achieving a subsequent pregnancy. Although the mechanism that insertion of Cu-IUD before embryo transfer increase implantation rate is not clear, it is possible that Cu-IUD stimulates endometrial inflammation and the change of inflammatory factors favours embryo implantation.

Limitations, reasons for caution: The current findings support a possible role of hysteroscopy and Cu-IUD in treating women with RIF. Further large randomized controlled trials are required to investigate the efficacy of this intervention. The mechanism need to be investigated further.

Wider implications of the findings: Pregnancy outcomes are improved in women with repeated implantation failure (RIF) by office hysteroscopy and placing copper intrauterine device (Cu-IUD). This means may be a good alternative approach for RIF.

Trial registration number: NO

P-778 Clinical efficacy of modified adenomyomectomy in infertile women with adenomyosis

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Study question: Does surgical removal of adenomyosis have clinical efficacy in infertile women?

Summary answer: Modified adenomyomectomy as a uterus-sparing surgery could be an effective method for increasing pregnancy rate and conservation of fertility potential in infertile women with adenomyosis.

What is known already: The relation between infertility and uterine adenomyosis is controversial, but it appears to affect endometrial receptivity and increase abortion rate. In infertility where uterine conservation is paramount, the treatment of adenomyosis is often complicated, that is to say, medical treatment is often transient and hysterectomy for eradication could not preserve their fertility. At this stage, there is no agreement on the most appropriate therapeutic methods on fertility outcome in infertility patients with adenomyosis. Regarding surgical removal of adenomyosis, including laparoscopic reduction, uterus-sparing surgery appears to be satisfactory and reduced the need for hysterectomy, but needs well designed prospective study.

Study design, size, duration: Prospective clinical trial was conducted. The subjects consisted of 41 infertile patients with adenomyosis and were enrolled after the failure of *in vitro* Fertilization (IVF) for pregnancy from December 2007 to August 2015.

Participants/materials, setting, methods: All cases were classified as having unexplained infertility, adenomyosis with severe periodic dysmenorrhea and occasional menorrhagia. This newly designed operative procedure included pediatric foley insertion into the uterine cavity, injection of diluted vasopressin along the uterine incision, T- or transverse H-incision on the adenomyotic wall, careful excision of adenomyosis tissue using argon laser under intra-operative ultrasonography. After reduction surgery, patients underwent follow up examination for symptom relief, reduction of adenomyosis by MRI and pregnancy rate.

Main results and the role of chance: The mean age and the duration of infertility were 35.07 ± 3.07 years and 57.12 ± 49.93 months, respectively. The mean volume of excised specimens of adenomyosis was $94.40 \pm 61.79g$. The relief of dysmenorrhea was observed clearly in all patients at 6 months after operation (NRS; 7.07 ± 2.50 vs. 1.07 ± 0.83, $p < 0.001$). The amount of menstrual blood was also significantly decreased (137.50 ± 97.56 vs. 72.75 ± 67.32, $p = 0.023$). The CA 125 level was significantly decreased at the time of 6 months after operation (187.75 ± 229.52 vs. 20.36 ± 19.19, $p = 0.026$). Post-operational complication occurred in four patients (hematoma, ureter fistula, shrinkage of uterus and primary ovarian insufficiency). Six patients were lost in the follow-up. Of 28 patients who attempted pregnancy, 16 patients conceived by IVF-ET or T-ET after the operation (16 of 28; 57.1%). However, miscarriage occurred in four patients, ectopic pregnancy in three patient, preterm delivery in one patient and eight patients (8 of 28; 28.6%) delivered by cesarean section at term. The rest of the patients have been trying to conceive by IVF-ET or natural course.

Limitations, reasons for caution: The sample size was small, so further study with larger number of patients will be helpful to investigate the possibility of this result.

Wider implications of the findings: This modified adenomyomectomy was related to symptom relief of dysmenorrhea, menorrhagia and increasing pregnancy rate, implying that reduction surgery could be considered as a successful method for infertile women with adenomyosis who need fertility preservation. This is the first report on the clinical pregnancy outcome of uterus-sparing surgery in adenomyosis.

Trial registration number: N/A

P-779 The optimal number of embryo transferred for patients with didelphys uterus: following 115 cases after *in vitro* fertilization embryo transfer (IVF-ET)

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Study question: To investigate possible optimal number of embryo transferred for didelphys uterus according to reproductive outcomes between singleton and twins pregnancies in this uterine morphology.

Summary answer: One good quality embryo transfer is recommended for didelphys uterus since twin's pregnancies dramatically increase risks of prematurity and low birth weight.

What is known already: Most literature on didelphys uterus were based on small sample or case studies which indicated prematurity rate was relatively higher in didelphys uterus compared with normal uterus. However, no studies analyzed the differences of reproductive outcome between singleton and twins pregnancy in didelphys uterus and investigated whether risks of obstetric outcome come from twins pregnancies to some extent because this anomaly were frequently accompanied with relatively small uterine cavity. No studies discussed the strategy on the number of embryo transferred to reduce these risks. Besides, infertile female have higher prevalence of didelphys uterus compared with the unselected population.

Study design, size, duration: From March 2003 to April 2015, 115 cases with didelphys uterus who experienced IVF-ET were retrospectively analyzed. There

were 76 cases achieved clinical pregnancy. One case had ectopic pregnancy. One case had triple pregnancy and two embryos demised spontaneously. Seven cases had twin's pregnancy firstly and then one embryo demised spontaneously. The rest cases were divided into singleton (52 cases) and twins (15 cases) pregnancy group according to the pregnancy number.

Participants/materials, setting, methods: Only good quality embryos were transferred including day 3 embryos which majority blastomere are regular size, fragmentation < 20% and blastosphere whose grade above 3BB (Gardner grading system). Comparison of pregnancy outcome, preterm delivery rate, cesarean section rate and birth weight were made between singleton and twins pregnancy groups. The clinical pregnancy rate and the number of gestation were calculated and recorded based on different numbers of embryo transferred.

Main results and the role of chance: The clinical pregnancy rate for patients with didelphys uterus was 66.1% (76/115). The miscarriage rate in first trimester (3.9% (2/52) vs. 0% (0/15)), second trimester (1.9% (1/52) vs. 6.7% (1/15)) and the live birth rate (94.2% (49/15) vs. 93.3% (14/15)) between singleton and twins pregnancy groups were not significantly different ($P = 0.483$). The cesarean section rate were both 85.71% in singleton (42/49) and twins (12/14) groups. However, the prematurity rate in twins pregnancy was significantly higher compared to singleton pregnancy (64.3% vs. 22.4%, Odds ratio: 6.21; 35.5 ± 2.7 vs. 38.3 ± 0.6 weeks of gestation). What is more, the birth weight in twins group was lower compared with that in singleton group (2.23 ± 0.46 vs. 3.11 ± 0.55 Kg). In twin's pregnancy group, there were two infant with perinatal death caused by pulmonary hypoplasia. When one (15 cases), two (73 case) or three (27 case) embryos were transferred, the clinical pregnancy rate were 66.7%, 65.8% (42.5% single pregnancy and 23.3% twins pregnancy) and 66.6% (40.7% single pregnancy, 22.2% twins pregnancy and 3.7% triple pregnancy) respectively.

Limitations, reasons for caution: Seven cases with twin's pregnancy had spontaneously one fetus demise but the pregnancy outcome, reason and potential clinical implication of one fetus demise were not analyzed. Further studies will be conducted on this phenomenon to figure out potential implication on the number of embryo transferred in uterine anomaly population.

Wider implications of the findings: One good quality embryo transfer is recommended for patients with didelphys uterus even probably for other uterine anomaly because one good quality embryo transfer would achieve the similar clinical pregnancy rate compared with multiple embryo transfer and reduce risks of prematurity and low birth weight from multiple pregnancy.

Trial registration number: None

P-780 Histeroscopic treatment for infertility women: Myosure

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Study question: The objective of this manuscript is to evaluate the fertility results and pregnancy outcomes in infertile women after treatment of intrauterine pathology with de Myosure tissue removal system.

Summary answer: Post-operative fertility was higher in women who suffered from myoma type 0 and was lower in women whose largest and intramural component.

What is known already: The association of subserous fibroids and intramural fibroids with infertility is controversial. But the role of submucous fibroids which are reported in 5%-18% of patients as a causal factor for infertility is likely.

A few retrospective studies published in 1990s have demonstrated successful reproductive outcome after hysteroscopic removal of submucous myomas in infertile women.

Study design, size, duration: A retrospective study was performed from June 2014 through September 2015. A total of 150 patients had undergone hysteroscopic myomectomy during the study period. Of these, 8 patients who were subfertile and fulfilled our criteria were included.

Submucous fibroids were classified based on European Society of Gynaecological Endoscopy into type 0-whole of fibroid inside the uterine cavity. Type 1-less than 50% extension of submucous fibroid into myometrium. Type 2-more than 50% extension into myometrium.

Participants/materials, setting, methods: For each patient operated in a context of infertility, we analyzed ovarian function and partner's semen. We examinate the uterine cavity by diagnostic hysteroscopy or hysterosalpingography. The following collected data was characteristics of myomas; tube appearance, permeability, and aspect of the mucosa. The variables collected were the age, duration of infertility; existence of menometrorrhagia, existence of uterine cavity deformation; number of myomas; type, size and location of the largest myoma.

Main results and the role of chance: Histeroscopic Unit only admitted in this study myomas discovered during the work-up for infertility The mean patient age was 32 years (range: 24–40 years). The mean duration of infertility was 42 months (range: 12–108 months). The mean follow-up was 26 ± 10 months. A total of 6 patients were symptomatic (75%). The most common symptoms were menorrhagia which was present in seven patients and dysmenorrhea/pelvic pain in five patients. During the process 4 of 8 (50%) patients needed the second morcellation. In the classification 50% were type 0, 20% type I and 30% type II. In process 2 (25%) patients achieved spontaneous pregnancy, 3 become pregnant with assisted reproduction, 2 by insemination and other by *in vitro* fecundation; who obtained a cornual pregnancy resolved through surgery and finally successful intrauterine pregnancy. Other 37% was until waiting to get the pregnancy. The average diameter of pathology removed was 3±0.7 cms for fibroids.

Limitations, reasons for caution: Possible biases related to retrospective studies and limited number of cases.

Wider implications of the findings: This investigation reveals the myosure as a technique histeroscopic to solve the uterine patology. The limited cases not allow to get important results. But myosure begin to appear as a alternative from the surgical procedure in the patients.

Trial registration number: Funding by Hospital. Hospital CUHU, Huelva, Spain.

P-781 Frequency of endometrial cancer and atypical hyperplasia in infertile women undergoing hysteroscopic endometrial polypectomy

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Study question: What is the frequency of endometrial cancer (EC) patients among infertile women undergoing hysteroscopic endometrial polypectomy due to endometrial polyps?

Summary answer: EC or atypical hyperplasia (AH) is found in 0.95% of infertile women undergoing hysteroscopic endometrial polypectomy with endometrial polyp.

What is known already: In Japan, the incidence of EC is reportedly ≤0.003% in women between 30 and 39 years old. The high-risk group for developing uterine EC shows an association with infertility. Endometrial polyps cause implantation disturbance, and so are usually resected irrespective of size. Two popular methods are used for extraction of endometrial polyps: blind dilatation and curettage; and hysteroscopic polypectomy. In general, blind dilation and curettage can miss endometrial pathology in approximately 50% of cases, so hysteroscopic polypectomy remains the gold standard for both diagnosis and treatment of endometrial polyps.

Study design, size, duration: A total of 1,049 infertile patients who underwent office-based hysteroscopic polypectomy at Sugiyama Clinic Marunouchi between July 2011 and October 2015 were eligible for this retrospective study. All patients had been diagnosed with endometrial polyp via hysterofiberscopy prior to operation.

Participants/materials, setting, methods: All patients underwent hysteroscopic endometrial polypectomy using a resectoscope (OES Pro, Olympus, Japan) with monopolar resection. Surgical specimens were examined histopathologically. Characteristics of patients diagnosed with EC from histopathological examination were evaluated retrospectively.

Main results and the role of chance: Median age of patients was 32 years (range, 19–50 years). According to histopathological examination, EC was found in 10 patients (incidence, 0.95%). The histological type of EC was as follows: endometrioid adenocarcinoma G1; endometrioid adenocarcinoma G2; endometrioid adenocarcinoma G3; and atypical hyperplasia.

Type of EC	Incidence, *n*
Endometrioid adenocarcinoma G1	3
Endometrioid adenocarcinoma G2	1
Endometrioid adenocarcinoma G3	2
Atypical hyperplasia	4

Median age of EC patients was 34 years (range, 28–41 years), and median body mass index (BMI) was 21.2 kg/m^2 (range, 16.7–29.9 kg/m^2). Nine EC patients were nulliparous, and all had undergone infertility treatment, with only one woman having delivered healthy babies. Ovulation disorder was noted in four patients, and obesity (BMI > 25 kg/m^2) in only one. Polycystic ovaries (PCO) were concomitantly observed in one patient. However, abnormal vaginal bleeding was not noted in any patients.

Limitations, reasons for caution: The incidence of EC in this study was markedly higher than that in the Japanese population. All patients in this study had undergone infertility treatment, and infertility itself represents a risk factor for developing EC. Several biases might thus have contributed to this incidence.

Wider implications of the findings: Hysteroscopic polypectomy should be performed when endometrial polyps are detected on investigational screening, and surgical specimens should be checked for the presence of malignancy.

Trial registration number: This study does not have RCT status, and therefore did not receive a trial registration number.

P-782 Three-month treatment with ulipristal acetate prior to laparoscopic myomectomy of large uterine myomas

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Study question: Which is the usefulness of preoperative treatment with ulipristal acetate (UPA) in patients undergoing laparoscopic excision of large uterine myomas?

Summary answer: A 3-month treatment with UPA prior to laparoscopy for large uterine myomas decreases intraoperative blood loss, hemoglobin drop, postoperative blood transfusion and length of surgery.

What is known already: Randomized controlled trials showed the efficacy of UPA in decreasing the volume of uterine myomas, in improving menorrhagia and iron-deficiency anemia. No previous study investigated the use of preoperative UPA in patients undergoing laparoscopic excision of uterine myomas.

Study design, size, duration: This was a retrospective analysis of a prospectively collected database. The study included all consecutive patients who underwent laparoscopic myomectomy at our Institution because of heavy menstrual bleeding caused by large uterine myomas. Patients included in the study underwent either direct surgery (group S, $n = 43$) or received 3-month preoperative treatment with UPA (5 mg/day orally, Esmya; Gedeon Richter, Budapest, Hungary; group UPA, $n = 34$).

Participants/materials, setting, methods: The study included premenopausal women undergoing laparoscopic myomectomy because of FIGO type 3, 4 or 5 myomas with largest diameter ≥10 cm and total number of myomas ≤3. The primary objective was to evaluate whether UPA decreases intraoperative blood loss. Secondary objectives were to compare changes between study groups in operative outcomes, incidence of complications, and to report adverse events, changes in preoperative hemoglobin levels and myoma volume caused by UPA.

Main results and the role of chance: Mean (±SD) intraoperative blood loss was lower in group UPA (504.7 ± 212.2 ml) than in group S (686.2 ± 315.6 ml; $p = 0.012$). The operative time was lower in group UPA (137.6 ± 26.8 min) than in group S (159.7 ± 26.8 min; $p < 0.001$) while there was no significant difference in the suturing time between the two study groups ($p = 0.076$). Hemoglobin drop was lower in group UPA (1.1 ± 0.5 g/dl) than in group S (1.3 ± 0.7 g/dl; $p = 0.034$). Six patients in group S and no patient in group UPA required postoperative blood transfusion ($p = 0.031$). Complications (hematoma, fever >38°C, urinary tract infection) were infrequent and not significantly different between the two groups ($p = 0.726$). Preoperative 3-month treatment with UPA caused a significant increase in hemoglobin levels (11.9 ± 1.6 g/dl) compared with baseline (9.1 ± 1.1 g/dl; $p < 0.001$) and a 31.8% (±10.7%) decrease in total myoma volume.

Limitations, reasons for caution: Firstly, this research is limited by the retrospective study design. Secondly, UPA was not compared with other hormonal therapies commonly used for the preoperative treatment of uterine myomas, such as GnRH analogues. Thirdly, the surgeons performing laparoscopic myomectomy were not blinded to the treatment received by the patients.

Wider implications of the findings: This study shows that a 3-month preoperative treatment with UPA before laparoscopic myomectomy for large uterine myomas is useful to reduce intraoperative blood loss, operative time, haemoglobin drop and the need for blood transfusions. Future studies should compare UPA with other preoperative standard treatments such as GnRH analogues.

Trial registration number: Not applicable.

P-783 Laparoscopic guided tubal catheterisation for proximal fallopian tube occlusion. A retrospective cohort study

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Study question: What are the reproductive outcomes of infertile women with proximal tubal occlusion (PTO) following laparoscopy-guided hysteroscopic tubal catheterisation?

Summary answer: Women with proximal tube occlusion treated with laparoscopy-guided hysteroscopic tubal catheterisation at the Birmingham Women's Hospital have a 27.8% chance of natural pregnancy.

What is known already: Tubal disease is the cause of infertility in 30% of women unable to conceive naturally. The cause of tubal infertility can be infection, previous pelvic surgery and endometriosis. Prior to the widespread availability of *in vitro* fertilisation (IVF), tubal surgery was commonly performed. In the case of proximal tubal occlusion (PTO), tubal catheterisation (TC) was attempted. However, due to increase reliance on IVF, the availability of TC is now limited to selected gynaecology centres in the UK. In the UK, government funded IVF cycles are limited therefore TC remains an important management strategy.

Study design, size, duration: This is a retrospective cohort study of women with PTO as their main cause of infertility. The cohort underwent laparoscopy-guided hysteroscopic tubal catheterisation between March 2010 and March 2015. A postal survey was sent to all women in October 2015 to ascertain their reproductive outcomes following tubal catheterisation.

Participants/materials, setting, methods: Fifty-four women with PTO as the main cause of infertility underwent tubal catheterisation in a large teaching hospital between March 2010 and March 2015. Diagnosis of proximal tubal occlusion was confirmed pre-operatively by hystero-salpingogram or laparoscopy and dye testing. Demographic information and operative details were reviewed. All women were invited to complete a questionnaire regarding natural conception, need for assisted reproductive treatment and other reproductive outcomes.

Main results and the role of chance: The 54 women in our cohort study had a mean age of 32.9 years (SD 5.9) and Body Mass Index of 27.2 (SD 4.9). Twenty-three women (42.6%) had primary infertility and 31 women (57.4%) had secondary infertility. Thirty-two of the 54 women (64.8%) had unilateral tubal blockage and 22 (35.2%) had bilateral blockage. In 31 out of 54 (57.4%) women, successful catheterisation was demonstrated by a positive tubal dye test at the end of tubal catheterisation. No surgical complications were identified in the clinical records. Postal surveys were sent to all 54 women, 36 women responded (66.7%) and provided data regarding reproductive outcomes. The intra-operative catheterisation success rate was similar for those that responded to the survey and those that did not (55.5% vs. 57.4%, $p = 0.876$). Natural clinical pregnancy occurred in 10 out of 36 respondents (27.8%), with a live birth rate of 6 out of 36 respondents (16.7%). The median interval from tubal catheterisation to spontaneous conception was 197 days (IQR 91-273). Four women out of 10 achieving natural clinical pregnancy miscarried but none had an ectopic pregnancy. Fourteen of the 36 respondents required IVF treatment, with seven achieving a subsequent live birth.

Limitations, reasons for caution: This is a small cohort study of 54 women so the results must be interpreted with caution. Although the Birmingham Women's Hospital covers a diverse population the generalisability of the findings may be limited as this was a cohort study from a single centre.
Wider implications of the findings: In the UK, government funding for IVF is limited. In women who have PTO who have difficulty in self-funding multiple IVF cycle attempts, surgery for tubal pathology is an important alternative management strategy. Tubal catheterisation provides a low risk treatment option achieving almost comparable clinical pregnancy rates to IVF.
Trial registration number: The study was approved by the UK National Research Ethics Service Committee West Midlands – Black Country (15/WM/0199).

POSTER VIEWING SESSION

STEM CELLS

P-784 Human spermatogonial stem cells survive but show limited proliferation rate *in vitro* under mouse spermatogonial stem cell culture conditions

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Study question: Are human Spermatogonial Stem Cells (hSSCs) able to proliferate *in vitro* in co-culture with inactivated somatic feeder cells under mouse SSC (mSSCs) culture conditions?
Summary answer: hSSCs are able to survive but do not proliferate *in vitro* in co-culture with inactivated somatic feeder cells under mSSC culture conditions.
What is known already: Previous studies based on conditions already employed for mSSC *in vitro* propagation and the different attaching properties of somatic and germ cells to create an *in vitro* system for the long term *in vitro* propagation of hSSCs. However, a recent report detected a limited ability of human germ cells to survive *in vitro* beyond 1 month and hypothesized that this was due to the higher proliferation rate of the somatic fraction. On the other hand, some reports indicate that human testicular cells sorted for the markers EPCAM+/HLA-, results in an enriched hSSC population.
Study design, size, duration: Time course study at 7, 14 and 28 days comparing the *in vitro* appearance, phenotype, and propagation rate of hSSCs. Testicular tissue from 3 human donors with prostate cancer was used to create *in vitro* cell cultures corresponding to the following treatment groups: 1) Unsorted human testicular cells, 2) Differentially plated human testicular cells, and 3) EPCAM+/HLA-enriched testicular cells in co-culture with inactivated testicular feeders from the same patient.
Participants/materials, setting, methods: Cryopreserved testicular tissue from human adult male donors with prostate cancer. Testicular cells obtained by mechanical/enzymatic digestion were FAC-Sorted and co-cultured with testicular feeder cells previously irradiated at 50 Gy. Analyses and characterization included hormone production/intake measurement, immunocytochemistry, TUNEL and RT-qPCR.
Main results and the role of chance: Putative hSSCs appeared in singlets, doublets or small groups of up to 8 cells *in vitro* only when testicular cells were cultured in StemPro medium supplemented with GDNF, LIF, bFGF and EGF. FAC-Sorting based on the EPCAM+/HLA-phenotype resulted in an enrichment of 27% VASA+/UTF1+ putative hSSCs, compared to 13% in unsorted controls. Co-cultured sorted cells with inactivated testicular feeders gave rise to a relative enrichment of hSSCs with an average density of 173 hSSCs/cm^2 after 2 weeks *in vitro*, compared to the culture of unsorted testicular cells (83 hSSCs/cm^2) and differentially plated testicular cells (50 hSSCS/cm^2). However, putative hSSCs rarely stained positive for the proliferation marker Ki67 and their presence was reduced until almost dissapear after 4 weeks *in vitro*. Sorting EPCAM+/HLA- cells and co-culturing with inactivated testicular feeders improved the germ cell/somatic ratio *in vitro* compared with previous reports. The mouse-based culture conditions supported the survival of hSSCs, but not their proliferation. This phenomenon is similar to the *in vivo* situation where the mouse testicular niche allows the survival but not the proliferation nor the maturation of hSSCs transplanted into the mouse seminiferous epithelium.
Limitations, reasons for caution: This study was performed with frozen/thawed testicular tissue from three aged donors with normal spermatogenic histology. However, a possible bias on the results due to the age of the donors cannot be totally discarded.
Wider implications of the findings: Fertility preservation strategies in boys include the amplification of hSSCs. Our results indicate that the already reported methodology for hSSC *in vitro* propagation is not sufficient to induce hSSC proliferation *in vitro*. Further research on the needs of hSSCs for their *in vitro* propagation is mandatory.
Trial registration number: Not applicable.

P-785 Transplantation induced pluripotent stem cells in situ improves fertility outcome impaired by intrauterine adhesions in mice

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Study question: To investigate the therapeutic potentials of induced pluripotent stem cells(iPSCs) to treat intrauterine adhesions(IUA) and further explore the possible mechanism involved.
Summary answer**:** iPSCs may be a pragmatic solution for the treatment of IUA and heme-oxygenase-1(HO-1) gene plays an important role in regulating the therapeutic effect of iPSCs.
What is known already: Traditional treatment nowadays for IUA can only restore the shape of the uterine cavity but it remains difficult to repair the inadequate endometrium. While the application of iPSCs is widely popularized in many degenerative diseases by the means of differentiating stem cells into different cell types, its usage in treating IUA is still fragmentary.
Study design, size, duration: A cross sectional study was carried out to compare the efficacy of different treatment modalities in mouse IUA model among 5 groups (20 mice/each). Five mice from each group were sacrificed at various times following treatment (24h, 5d), in order to evaluate the change of acute and chronic inflammation on endometrium. The rest mice (n = 10 in each group) were mated with male mice to observe the subsequent pregnancy rate 28d after treatment.
Participants/materials, setting, methods: A mouse IUA model was established with dual injury of lipopolysacchairdes and mechanical injury. Different cells were infused in situ into the mice uterus after modeling, including PBS, MEF, iPSCs, iPSCs-ho1siRNA and iPSCs-CTsiRNA. Subsequent fertility outcomes were observed 28d after treatment, so were the degree of tissue edema and inflammation 24h and 5d after treatment. The presence of iPSCs was demonstrated by immunofluorescence and ER expression of the impaired endometrium was also detected by IHC.
Main results and the role of chance: Transplantation iPSCs in situ could significantly increase pregnancy rate and fetus number when compared with controls(PBS and MEF) ($P < 0.05$). Pathological studies showed that iPSCs could significantly ameliorated tissue edema and inflammation with the proof of having higher number of nucleus but less neutrophil than control groups 24h after treatment($P < 0.05$). Furthermore, iPSCs were detected in the impaired endometrium with red fluorescence 5d after treatment. This, coupled with the increased level of ER expression and number of endometrial gland, strongly indicated the functional improvement of impaired endometrium. However, when knocking down HO-1 expression, the effect of iPSCs was significantly weakened ($P < 0.05$).
Limitations, reasons for caution: As an animal study, the mouse model couldn't simulate all causes of IUA from the patients. The safety of offspring and the exact mechanism of how to effectively direct differentiating iPSCs to hormonal responsive endometrial cells are also needed to be delineated before clinical translation.
Wider implications of the findings: Our study highlights the potential role of iPSCs in promoting endometrial regeneration, which may offer the possibility not only to treat refractory IUA but also prevent IUA after uterine injury.
Trial registration number: None.

P-786 Identification of functional integrin heterodimers expressed on spermatogonial stem cells in mice

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Study question: Which kind of integrin heterodimers was expressed on plasma membrane of mouse spermatogonial stem cells?
Summary answer: Mouse spermatogonial stem cells have integrin α6β1 and αVβ1 on plasma membrane.
What is known already: Generally, fate of spematogonial stem cells (SSCs) can be determined specifically by microenvironments surrounded with various extracellular matrix (ECM) components and integrins recognizing directly ECM proteins play an important role in transporting ECM-derived signals into cytoplasm, resulting in inducing a variety of biological functions such as cell attachment, self-renewal and differentiation. However, to date, studies on type of functional integrin heterodimers expressed on the undifferentiated SSCs remain unclear.
Study design, size, duration: We tried to investigate systematically what kind of integrin heterodimerssubunits are expressed transcriptionally, or translationally and functionally in the spermatogonial stem cells derived from testis of ICR mouse.
Participants/materials, setting, methods: Isolation of spermatogonial stem cells from testis were conducted by magnetic activated cell sorting (MACS) using Thy1 antibody. Subsequently, transcriptional and translational level of integrin α and β subunits in the isolated spermatogonial stem cells were measured by real-time PCR and fluorescent immunoassay, respectively. Moreover, functionality of presumed integrin heterodimers was confirmed by antibody inhibition assay.
Main results and the role of chance: Transcriptional levels of genes encoding total 25 integrin subunits were quantified, and integrin α1, α5, α6, α9, αV and αE and integrin β1, β5 showed higher expression levels than other subunits. In contrast, integrin α2, α3, α4, α7, α8, α10, αL, αM, αD, and αX, and integrin β2, β4 and β7 were weakly transcribed. When translational levels of the integrin α subunits showing high transcription level (α1, α5, α6, α9, αV and αE) were measured, integrin α5, α6 and αV were higher than integrin α1, α9 and αE. In case of integrin β subunit, β1 evaluated more expression than β5. Based on these results, we speculated that the undifferentiated spermatogonial stem cells derived from ICR mouse might express integrin α5β1, α6β1 and αVβ1 on plasma membrane. Subsequently, functional blocking of integrin α6β1 or αVβ1 in SSCs significantly inhibited attachment to laminin or vitronectin, whereas functional blocking of integrin α5β1 did not inhibit significantly attachment to fibronectin.
Limitations, reasons for caution: The results of this study was derived from only mouse with outbred strain. Therefore, studies on the expression of integrin hetrodimers in different strain mouse, other species and human should be conducted in the future.
Wider implications of the findings: This information will greatly contribute to constructing non-cellular niche supporting self-renewal of SSCs in the future.
Trial registration number: –

P-787 Study of the epigenetic cellular memory induced by morphine upon Epiblast-like cells (EpiLCs) differentiation

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Study question: To analyze the genetic expression changes induced by morphine during EpiLCs differentiation and to determine its epigenetic cellular memory
Summary answer**:** The Polycomb/H3K27me3 complex seems to be involved in the epigenetic memory induced by morphine, causing an inhibition of male reproductive processes during EpiLCs differentiation.
What is known already: The epiblast is derived from the inner cell mass in the mammalian blastocyst giving rise to the three primary germ layers: the ectoderm, the endoderm and the mesoderm. If there is a presence of external stimuli, in contact with the blastocyst before or during epiblast cells differentiation, it might affect to the developmental process. Due to the fact that morphine is the most active component of opium and its therapeutic value for pain relief, our aim is to elucidate the effect of morphine on differentiation and to identify the existence of a cellular memory produced by morphine upon EpiLCs differentiation.
Study design, size, duration: mESC, 24 h morphine treatment, *in vitro* EpiLCs differentiation (using activin A, bFGF and KSR).
Participants/materials, setting, methods: High density microarray, qRT-PCR, immunoblotting approaches.
Main results and the role of chance: EpiLCs where differentiated (Hayashi et al., 2011) from morphine treated and untreated mESC. After 24h morphine treatment we have evidenced a lower mESC cell amount, without altering the viability and the pluripotent status, suggesting that morphine affects the cellular cycle. We have carry out a transcriptome analysis by the microarray technique, to identify morphine target processes, in mESC, and EpiLCs. Specifically, 87 genes related to the function of the mESC cell membrane were altered in these cells. On the other hand, there was a 1,462 genes deregulation in EpiLCs, suggesting the existence of a cellular memory between mESC and EpiLCs. The ontology analysis on EpiLCs derived from morphine treated mESC, showed that there is down-regulation on genes associated to male reproductive processes, while those related to neural development functions are up-regulated. The transcriptome and qRT-PCR analysis also showed a down regulation on Ezh2 gene, which belongs to the epigenetic regulator Polycomb Complex, associated with inactive genes through the H3K27 histone methylation. Therefore, we have verified the down regulation of H3K27me3 in morphine treated mESC, by immunoblotting, suggesting that the complex Polycomb/H3K27me3 can be involved in the epigenetic memory induced by morphine during EpiLCs differentiation.
Limitations, reasons for caution: Complex data analysis by bioinformatic resources.
Wider implications of the findings: The potential implication of H3K27me3 in the epigenetic memory induced by morphine in differentiated EpiLCs, offers a new target to establish the basic knowledge to understand the mechanisms underlying the epigenetic cellular memory. The fact that reproductive processes are affected by morphine provides possible evidences for a transgenerational epigenetic inheritance.
Trial registration number: No trial registration number

P-788 Construction of endometrial-like epithelium using human endometrial CD146 positive cells *in vitro*

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Study question: Does human endometrial CD146$^+$ mesenchymal stem cells could construct endometrial-like epithelium *in vitro*?
Summary answer: The CD146 cells derived from human endometrium have the potential tobe used for *in vitro* construction of endometrial-like epithelium in a three-dimensional (3D) culture system
What is known already: It has been proposed that a 3D culture system using CD146$^+$ endometrial stem cells on collagen/matrigel scaffold and uterine smooth muscle cells could construct endometrial-like epithelium.
Study design, size, duration: This laboratory-based study used human endometrial and myometrial tissues from women at proliferative phase aged 30–40 years (n = 10) undergoing hysterectomy for non-endometrial and -myometrial pathologic conditions.

Participants/materials, setting, methods: Human endometrial cells were cultured to the fourth passage, then CD146+ cells were sorted using MACS. Then they were cultured in collagen/matrigel scaffold on the top of the myometrial smooth muscle cells for 10 days. At the end of culture period, the differentiation and formation of the epithelial-like cells were confirmed by ultrastructural studies and analysis of the expression of SPP1, MMP2, ZO-1, LAMA2 and COL4A1 genes by RT-PCR and CD9 protein by western blotting.
Main results and the role of chance: The results showed that the human endometrial mesenchymal CD146+ cells were able to produce endometrial glandular tube-like structures *in vitro*. Ultrastructural observation revealed the polarization of cells as epithelium by appearance of some projections on the apical basal lamina-like structures on the basal and tight junctions and desmosomes on the lateral surfaces of the epithelial-like cells. The expression of SPP1, MMP2, ZO-1, LAMA2 and COL4A1 gens and CD9 protein confirmed the formation of endometrial epithelial-like cells.
Limitations, reasons for caution: This study does not demonstrate the functional properties of the formed epithelial-like cells, it needs more study to evaluate the interaction of embryo with this endometrial like cells.
Wider implications of the findings: This is the first attempt to construct endometrial-like epithelium in a 3D culture system using human endometrial CD146+ mesenchymal stem cells. This 3D culture system may have important implication in cell therapy and in human implantation models studies.
Trial registration number: It is not a clinical trial

P-789 Uncovering the novel role of mTORC2 subunit of the PI3K/AKT/mTORC pathway in establishing naive pluripotency in human embryonic stem cells

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Study question: How does the PI3K/AKT/mTORC pathway facilitate the induction of naive pluripotency in human embryonic stem cells (hESCs) converted in different naive conversion media?
Summary answer: Induction of naive pluripotency in primed hESCs occurs via activation of the mTORC2 subunit of the PI3K/AKT/mTORC complex, irrespective of the naive medium utilized.
What is known already: Efforts have been made to establish naive pluripotency in hESCs, such as by using naive conversion medium (NCM), naive human stem cell medium (NHSM) or reverse-toggle protocol (RT). Other published naive media rendered naive hESCs karyotypically abnormal or required ectopic transfection prior to exposure to the naive culture medium. Although the inhibition of MAPK/ERK, GSK3β and activation of LIF/STAT signalling is necessary to induce naive state, we recently demonstrated a novel role of PI3K/AKT/mTORC in naive hESCs converted in NCM media. However, the role of PI3K/AKT/mTORC in naive hESCs generated by other naive media has not been investigated so far.
Study design, size, duration: Unlike previous studies that compared different naive hESC conditions based on published datasets only, we carried out naive conversion of in-house derived primed hESCs using different naive media in the same lab, to avoid any bias. We induced the conversion of primed UG11-7 (XY) hESC line towards a naive state in NCM, NHSM or RT media in low oxygen conditions (5%) in the presence of mouse embryonic fibroblast feeder layer.
Participants/materials, setting, methods: Upon successful conversion of primed hESCs towards a naive state in NCM, NHSM and RT media, the naive hESCs from each condition were subjected to microarray analysis in biological and technical triplicates. The results were analyzed using the Qlucore Omics Explorer 3.1 software package. Genes were considered to be significantly differentially expressed if they had p-value ≤ 0.01 and fold change ≥|1.5|, i.e., expression levels between conditions had to be at least 50% different.
Main results and the role of chance: Genome-wide microarray-based transcriptional analysis revealed significantly increased expression of genes associated with mTORC2 complex including PROTOR1, MAPKAP1(SIN1), AKT1S1, DEPTOR and MLST8 in NCM-, NHSM- and RT-naive hESCs, whereas genes related to mTORC1 subunit were downregulated in these naive conditions. PROTOR1 is a known Rictor-binding subunit of mTORC2, facilitating activation of AKT1 via mTORC2 to aid in cell survival, further confirming the role of mTORC2 in inducing increased single cell clonogenicity in naive pluripotent hESCs (95%) compared to primed hESCs (25%). MAPKAP1 knockdown is known to be lethal to mouse embryo. AKT1 phosphorylation via mTORC2 facilitates cell proliferation and growth when expressed at higher levels compared to AKT2 and AKT3 which otherwise leads to mESCs differentiation. Remarkably, AKT1 continued to be highly expressed in all the three naive hESC conditions compared to AKT2 and AKT3, which further emphasizes the naive state of hESCs being induced via mTORC2 as the doubling time of naive hESCs, similar to naive mESCs, is reduced significantly (17 h) compared to primed hESCs (51 h). As well, AKT1 inhibits GSK3β via PI3K/AKT/mTOR activation. DEPTOR inhibition of mTORC1 leads to its overexpression on mTORC2, resulting in increased cell survival.
Limitations, reasons for caution: This study will benefit from in-depth transcription analysis for more naive hESC lines, male as well as female, to further validate the role of the PI3K/AKT/mTORC (mTORC2) pathway in naive pluripotency. Additionally, mechanistic studies involving the use of mTORC2-specific inhibitors and activators will also strengthen the novelty of our finding.
Wider implications of the findings: We demonstrate for the first time an unbiased approach to compare hESCs converted in different naive conditions by performing the study in one lab, using in-house derived hESCs. We reveal PI3K/AKT/mTORC (mTORC2) pathway as a novel naive pluripotency-related pathway in all naive culture media conditions.
Trial registration number: N/A

P-790 Inhibition of Rho-associated protein kinase (ROCK) in mouse embryos affects trophectoderm formation and blastocyst development, but still allows subsequent stem cell derivation

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Study question: Does Rho-associated protein kinase inhibition (ROCKi) affect the first lineage segregation in mouse embryos and still allow subsequent mouse embryonic stem cell (mESC) derivation?
Summary answer: ROCKi impairs trophectoderm (TE) cell formation in mouse embryos, still permitting successful mESC derivation. However, higher concentrations reduce both blastocyst development and subsequent mESC derivation.
What is known already: Hippo signaling regulates the first lineage segregation (TE and inner cell mass (ICM) formation) in mouse embryos. Yes-associated protein (YAP), a Hippo target, binds with Tead4 (TE marker) in nucleus, initiating Tead4 expression in the outer cells. YAP fails to enter nucleus in the inner cells repressing Tead4. ROCKi during mouse embryo culture activates Hippo signaling impairing TE proliferation and blastocyst formation. However, it is unknown whether mESCs can be derived from ROCKi embryos. From an ethical perspective, it would be valuable to enable ESC derivation from embryos that lack TE cells as they cannot develop further as individual.
Study design, size, duration: Zygotes were collected from B6D2/F1 mice. At two-cell stage, mouse embryos were exposed to ROCKi (Y-27632) (10 μM, 20 μM, 50 μM and 100 μM) and an equivalent amount of dimethylsulfoxide (DMSO) in controls. On the fourth day of treatment (~75h), blastocyst development was evaluated and all embryos (irrespective of whether they were blastocysts or not) were either fixed for immunostaining or plated for subsequent ESC derivation. This study was conducted in 295 two-cell stage mouse embryos.
Participants/materials, setting, methods: Blastocyst development from two-cell stage embryos was evaluated morphologically. Embryos were stained for Nanog (epiblast marker) and Cdx2 (TE marker). Positive cells were counted

using a Zeiss fluorescence microscope. mESCs were derived on gelatin-coated dishes using N2B27 medium supplemented with 1000U/ml leukemia inhibitor factor, 1 μM PD0325901 and 3 μM Chir99021. mESC derivation efficiency was measured based on the number of lines derived per total number of embryos plated. *P* values < 0.05 were considered significant.

Main results and the role of chance: Blastocyst development was similar to the control (95%) in presence of 10 μM (96.42%) and 20 μM (97.56%) ROCKi, but was severely inhibited in presence of 50 μM (21.42%) and 100 μM (0%) ROCKi (control: 85.36%). Most embryos that failed to form blastocysts displayed morula-like morphology and failed to form blastocoel cavity. Most embryos in 100 μM ROCK inhibitor had already started to degenerate on day 4. The number of Cdx2-positive cells (46.58 ± 14.91 (10 μM), 59.94 ± 11.04 (20 μM), 24.64 ± 16.14 (50 μM) and 10 ± 6 (100 μM)) was significantly reduced in embryos cultured in all concentrations of ROCKi compared to their respective DMSO controls (57.68 ± 13.19, 70.18 ± 14.77, 50.56 ± 21.16 and 50.56 ± 21.16). The number of Nanog-positive cells increased significantly with 10 μM (17.62 ± 5.39) and 20 μM (12.27 ± 4.95) ROCK inhibitor, whereas it remained unaffected with 50 μM (12 ± 6.90) and 100 μM (15.10 ± 5.53) ROCKi, compared to their respective DMSO controls (13.94 ± 4.22, 7.12 ± 2.44, 14.5 ± 5.29 and 14.5 ± 5.29). The total cell number was comparable in presence of 10 μM (74.87 ± 22.92) and 20 μM (83.33 ± 12.69) ROCKi (controls: 81.52 ± 15.33 and 81.5 ± 10.27). However, it was significantly reduced in 50 μM (41.17 ± 15.96) and 100 μM (30.47 ± 7.66) ROCKi (control: 72.18 ± 17.56). mESC derivation efficiency was 76.19% in control (no DMSO or ROCKi treated embryos) and 71.42% in 20 μM, 42.8% in 50 μM and 25% in 100 μM ROCK inhibitor treated embryos.

Limitations, reasons for caution: Although we found that ESCs could be derived from ROCKi treated mouse embryos showing no cavitation, it remains to be investigated whether those mESCs show similar self-renewal and pluripotent characteristics as mESCs derived from control embryos.

Wider implications of the findings: We found that ROCKi impaired TE formation in mouse, still yielding mESCs. As a next step, we will extrapolate these findings to human ESC derivation, where the derivation of hESC lines from non-viable embryos that lack TE cells and cannot form individual would be ethically less controversial.

Trial registration number: N/A

P-791 Assessment of pharmacological compounds during neural differentiation in human embryonic stem cells

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Study question: Human embryonic stem (hES) cells can be used in screening of neural embryotoxic chemicals.

Summary answer: Differentiated hES cells are useful for assessment of cell viability or neurogenesis impairment of chemicals.

What is known already: Human embryonic stem (hES) cells have been used in evaluation of embryotoxic chemicals because they are generated in large numbers and differentiate into three germ layers following formation of embryoid bodies. Using pluripotency of hES cells that have a potential for differentiation, they are currently used in assessment of toxicity of a variety of compounds.

Study design, size, duration: We examined the effects of several pharmacological compounds on hES cells differentiation into neuronal cells up to 28 days.

Participants/materials, setting, methods: Three anticancer agents (cytosine arabinoside, 5-fluorouracil, and hydroxyurea) and two immune suppressing agents (indomethacin and dexamethasone) were evaluated through cytotoxicity using CCK assay and changes of neural markers using real-time PCR and immunocytochemistry.

Main results and the role of chance: When three anticancer agents, cytosine arabinoside, 5-fluorouracil, and hydroxyurea, showed strong cytotoxicity, two immune suppressing agents, indomethacin and dexamethasone, showed a high concentration of $IC_{50,}$ which has weak cytotoxicity. Furthermore, most chemicals induced a significant change in levels of neuronal specific markers, such as *NR4A2* (dopaminergic neuron), *SLC1A2* (glutamartergic neuron), and *CNP* (oligodendrocyte). In addition, we monitored the neuronal lineage markers, including *FABP* (Neuronal progenitor), *FOXG1* (mortorneuron progenitor), *OLIG2* (oligodendrocyte progenitor), *GAD1* (GABAneuron terminal marker), and *SLC17A6* (glutamartergic terminal marker). We also found that cytosine arabinoside acts to diminish β-tubulin III in hES cells under conditions of neuronal differentiation because β-tubulin III is detected as a specific marker in extensive neurons.

Limitations, reasons for caution: We focused on the inhibited viability and the differentiation into cells of the neural lineage of human pluripotent stem cells by pharmacological compounds.

Wider implications of **the findings:** These findings could extend our understanding of how differentiated hES cells may be useful in assessment of cell viability or neurogenesis impairment of chemicals that could have an impact on the embryonic stage and its relevance in relation to embryonic neurogenesis.

Trial registration number: CBNU-201308-LRETC-008-01

P-792 Can Wnt inhibition induce more favorable characteristics in primed hESCs for future clinical applications?

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Study question: Can Wnt inhibition induce more favorable characteristics in primed hESCs for future clinical applications?

Summary answer: The Wnt inhibitor IWP2 converts hESCs into a distinct pluripotent state that displays more homogeneous pluripotent colony formation.

What is known already: HESCs hold great promise for future therapeutic applications. However, their primed nature is known for its heterogeneous expression of pluripotency markers, more spontaneous differentiation and low cloning efficiency. Recently, successes were obtained in converting hESCs into a more homogeneous, mESC-like (naive') pluripotent state, characterized by single cell clonogenicity. Yet, it remains uncertain whether or not this complicates directed differentiation. An alternative state has been described recently in mouse epiblast stem cells and hESCs using the Wnt inhibitor IWP2, exhibiting low levels of heterogeneity and spontaneous differentiation.

Study design, size, duration: Two in-house derived hESC lines, one male and one female, were converted towards IWP2 and naive conditions in three replicates. Standard primed hESCs were used as a control. Conversions were performed thrice from consecutive passages. After three passages, gene expression was analyzed by RT-PCR and immunofluorescence.

Participants/materials, setting, methods: HESC lines were cultured on mouse embryonic fibroblasts according to standard stem cell culture conditions. At three consecutive passages, cells were seeded into naive, IWP2 and primed conditions. After three passages, cells were fixed for immunofluorescence for pluripotency genes (POU5F1 and NANOG) and differentiation markers (GATA4 and T). RNA was collected from parallel cultures, for gene expression analysis of the same genes.

Main results and the role of chance: Overall, both IWP2-treated hESCs were more homogeneous than the parental primed hESCs. Levels of spontaneous differentiation were lower in IWP2-treated hESCs then in the parental primed hESCs, in all three replicates. Furthermore, IWP2-treated hESC colonies were distinct from both the naive and primed state hESC colonies. In the female line, the pluripotency marker genes POU5F1 and NANOG were not significantly different in the IWP2-treated hESCs compared to the primed condition. GATA4 and T were expressed in primed hESCs, but absent in IWP2-treated hESCs. In the male line, POU5F1 and NANOG were expressed similarly in primed and IWP2 conditions. However, GATA4 was expressed in primed hESCs, but absent in IWP2-treated hESCs, whereas T was not be detected in any condition.

Limitations, reasons for caution: Two different hESC lines were converted in triplicate, but we only assessed short-term effects of the IWP2 treatment of the pluripotency state. It will be important to investigate long-term effects as well. Moreover, directed differentiation experiments need to be performed to assess efficiency and possible clinical relevance of IWP2-converted hESCs.

Wider implications of the findings: In case the directed differentiation experiments are successful, the unique features of the IWP2 condition (homogeneous pluripotency nature and less spontaneous differentiation) will allow a new state of pluripotency that is perhaps more suitable for future clinical applications.

Trial registration number: N/A

P-793 PRD-like homeodomain transcription factor LEUTX has a central role in human embryonic genome activation (EGA)

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Study question: What is the expression profile and which are the target genes of human *LEUTX*?
Summary answer: Recently discovered new *LEUTX* isoform encodes functionally active homeodomain-containing transcription factor expressed exclusively in human embryos. The experimental *LEUTX* targets overlap with human EGA genes.
What is known already: PAIRED (PRD)-like homeobox genes belong to a class of predicted transcription factor genes with incompletely known functions. Our recent study of human EGA focusing on transcription start sites (TSSs) in oocytes, zygotes, and isolated blastomeres from 4-cell and 8-cell embryos (Töhönen et al., Nat Commun 6:8207, 2015) revealed that many PRD-like homeobox genes are uniquely expressed in the early embryos. One of the previously unannotated TSSs implicated in human EGA marked the predicted PRD-like homeobox gene *LEUTX*.
Study design, size, duration: We cloned the full-length *LEUTX* with a complete homeodomain from human 8-cell stage embryos. The gene was further cloned into a bicistronic pFastBac vector co-expressing eGFP marker and transfected to a human embryonal stem cell line. Nine to 11 h after transfection, we analyzed gene expression in 50-75 FACS sorted cells using single-cell tagged reverse transcription (STRT) RNA-seq.
Participants/materials, setting, methods: Human 8-cell stage embryos destined to be discarded were donated for research by informed consent. The human ESC lines HS401, HS980, HS983a and H9 were maintained in culture. Methods used included cDNA cloning, overexpression in human ES cells, FACS, STRT RNA seq, qPCR and database surveys.
Main results **and the role of chance:** Our results revealed that high *LEUTX* expression is restricted to early embryos only and we confirmed that mouse lacks the orthologous gene for human *LEUTX*. The novel *LEUTX* isoform containing a full homeodomain, but not the previously predicted *LEUTX,* activates more than 2,500 TSSs ($p < 0.1$) and the targets show remarkable overlap (FDR = 0.05, Chi-square test) with the genes that we previously identified in human EGA.
Limitations, reasons for caution: Due to the lack of mouse ortholog and the limited availability of human embryos, the functional studies in model systems are limited.
Wider implications of the findings: A novel *LEUTX* isoform is suggested to play an important role in early embryo development and pluripotency.
Trial registration number: Not available

P-794 Transcriptomic analysis of human primitive spermatogonia in adult testis

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Study question: We aim to examine the relationship between the differentiation stages of the human spermatogenesis and their transcriptomic activity in adult.
Summary answer: The dynamics of the germinal transcriptome has been observed along adult human spermatogenesis with a focus on primitive spermatogonia.
What is known already: Compared to mouse model, the identity of the stem cell pool in the human spermatogenic lineage is not well established. The molecular mechanisms governing human GSC germinal stem cell renewal and initiation of differentiation remain largely unknown.
Study design, size, duration: The various differentiation stages of human spermatogenesis were purified using a multi-parameter flow cytometry strategy with Vybrant, b-2microglobulin, a-6 integrin and Thy-1 markers. Using this strategy, three spermatogonial populations, one of which contains primitive spermatogonia and is enriched in spermatogonial stem cells, spermatocyte I, spermatocyte II and spermatids were sorted. A mix of somatic tissue was used as a reference. RNA was prepared and transcriptome was analysed using Affymetrix transcriptome array.
Participants/materials, setting, methods: Testicular biopsies were obtained from obstructive azoospermia patients exhibiting normal profiles of spermatogenesis in seminiferous tubules
Main results and the role of chance: According to PCA analysis, the transcriptome of the somatic reference is clearly distinct from transcriptome of the different germinal populations, showing the transcriptional specificity of the germinal lineage. We observe also a clear trend of the transcriptome to diverge according to the differentiation process. These comparisons reveal dramatic changes in gene expression profiles of each stage, exhibiting unique transcriptome profiles that underly the mechanistic differences between these steps. Premeiotic spermatogonial populations clustered closely together, and meiotic and post meiotic populations diverged. Transcriptomic signatures have been found for the different steps of spermatogenesis. We focused our analysis on the population of immature spermatogonia containing SSCs, and 551 genes (fold > 2, $p < 0.01$) were identified with enriched expression in this population compared with the downstream spermatogonial progeny.
Limitations, reasons for caution: Transcriptome
Wider implications **of the findings:** The characterization of the human spermatogonial stem cells and spermatogenesis should help to identify new molecular pathways and the genetic factors associated to infertility.
Trial registration number: Not applicable.

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