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ABSTRACT BOOK

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Short papers session 1A: ACE Pre-registration Jean Purdy Memorial prize - sponsored by CooperSurgical

SP1A.1 The impact of sequential and single-step media systems on embryo and blastocyst development, utilisation rates and clinical outcomes in ART

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Background: Media systems are broadly divided into two protocols: the sequential (SQ) approach (media formulation change on day 3) or the single-step (SS) 'let the embryo choose' approach (no change). The aim was to investigate differences in embryo development, quality, utilisation and biochemical and clinical pregnancy rates between Sydney IVF™ (Cook Medical, Australia) sequential and G-series™ (Vitrolife, Sweden) single-step media systems and to ascertain age-associated differences (<37yrs and ≥37yrs).

Methodology: Data were retrospectively collected from 137 patients (1422 oocytes) in SQ and 182 patients (2050 oocytes) in SS over consecutive 40-week periods. Fertilisation, early-cleavage (26hrs), days 2 (40-44hrs), 3 (64-68hrs) 5 (88-92hrs) and 6 (112-116hrs) development, quality and utilisation were assessed. Clinical outcomes were recorded. Fisher's exact test was used to ascertain statistical differences.

Results: There is a significantly higher fertilisation rate in SS ($P=0.0035$), and faster development at early cleavage check ($P=0.0003$), day 2 ($P<0.0001$) and day 3 ($P=0.0001$). Ideal-stage development was higher in SQ media at early-cleavage ($P=0.024$), day 2 ($P<0.0001$) and 3 ($P=0.0057$) significantly in the <37yr group. More blastocysts reached the ideal-stage of development in SQ on day 5 ($P=0.0009$) but not 6. Total proportion of blastocysts did not differ. Embryos were of a higher quality on day 2 ($P<0.0001$) and 3 ($P=0.0001$) in SQ media, but overall utilisation did not differ ($P=0.082$). More embryos were utilised in SQ media on day 3 in <37yr group ($P=0.019$), but not day 5 ($P=0.08$) or 6 (0.14). Biochemical and clinical pregnancy rates did not differ.

Conclusion: Sequential media appears to offer significantly better embryo developmental and quality, whereas single-step yields higher fertilisation rates. However, as utilisation and pregnancy rates did not differ significantly when patients were assessed cumulatively, the use of one media over the other to maximally benefit patients cannot be ascertained.

SP1A.2 The effect of multinucleation on pregnancy rates

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Background and objectives: Time-lapse technology has enabled the detection of irregular morphological events that occur in the embryo that cannot be detected by static observations. One of these events is multinucleation (MN). It has been suggested that MN embryos have an increased aneuploidy rate and low implantation rate. This retrospective study investigates whether the presence of the different types of MN (binucleation-BN, multinucleation-MuN and micronucleation-MiN) has an affect on implantation and pregnancy rates.

Methods: Biochemical, clinical pregnancy (CP) and implantation rates were compared in MN and non-multinucleated (NM) embryos. 433 IVF/ICSI cycles (501 embryos) cultured in the EmbryoScope time-lapse incubator (Vitrolife), between September 2016 and March 2018, were included in the study. MN was assessed at the 2 and 4 cell stage.

Results: The presence of MN was associated (though not significantly) with lower biochemical (37.6% vs. 44.9%, respectively), CP (26.7% vs. 33.7%, respectively) and implantation rate (24.4% vs. 31.6%, respectively) compared to NM embryos. When compared to BN embryos, MiN and MuN embryos were found to have lower biochemical (40.9% vs. 24.1%, 29.4%, respectively), CP (26.2% vs. 20.7%, 17.7%, respectively) and implantation rate (26.2% vs. 18.2%, 17.7%, respectively). Only the biochemical pregnancy rate in MiN embryos was significantly different ($P=0.031$). However, when combining the MiN and MuN groups together, both biochemical and implantation rates were significantly lower compared to NM embryos ($P=0.016$, 0.048, respectively). This suggests that the low sample size may have affected the significance of the results. Conclusion: MiN and MuN embryos have a reduced outcome compared to BN embryos. NM or BN embryos should be preferentially selected for transfer, when more than one embryo of the same stage and quality is available. Analysis of additional data, as well as extended analysis of other cycle variables will be performed in the future.

SP1A.3 Redefining blastocyst morphology to enhance efficacy of embryo selection and reduce inter- and intra-operator inconsistency

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Introduction: Blastocyst grading definitions vary between clinics. Even when using the same definitions, there is high inter- and intra- operator variability and low specificity of prediction of viability. Improvement of blastocyst morphology definitions in the context of time-lapse and artificial intelligence (AI) has the potential of improving consistency and efficacy of embryo selection. The objective was to identify quantifiable blastocyst image characteristics not currently in the Gardner grading definitions that (i) explain inter-/intra- operator variability in terms of consistencies and (ii) discrepancies, and (iii) predict live birth outcome.

Methods: Of the 395 blastocyst time-lapse images of known live birth outcome, 192 were included and the following parameters quantified: trophoctoderm (TE) number (total, TTN; and peripheral, PTN), individual TE cell width and height, blastocyst diameter. All images were assessed for expansion (1-6), inner cell mass (ICM) and TE morphological grade (Gardner grading, A-C) by five trained, independent embryologists. Parameters were compared with level of agreement, mode grades and live birth outcome.

Results: Several parameters explained consistencies (TE: TTN, Peripheral TE, Range and Mean TE width and diameter; Expansion: diameter, TTN, mean TE height) and discrepancies (TE: diameter, mean TE height; Expansion: peripheral TE; TTN) in grading by embryologists. TE grade ($p=0.019$) and TTN ($p=0.001$) were predictive of live birth outcome. The optimal threshold for defining embryos of good prognosis was $TTN > 37$ cells, cell width range < 75 pixels, and average cell width < 60 pixels.

Conclusion: Current definitions of blastocyst morphology are vague (using words like "few/many cells" and "tight/loose epithelium"). A revised definition of blastocyst morphology is proposed which includes quantifiable parameters assessing density and homogeneity of trophoctoderm cells aimed to reduce discrepancy and enhance efficacy of prediction. This improved grading definition is therefore proposed as more appropriate for embryo selection than the current Gardner grading.

SP1A.4 Differences in the timing of blastocyst development: Results from a prospective randomized comparative trial of human embryo culture media systems

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Background: A variety of commercially produced embryo culture media systems of differing composition are available. This study aimed to assess if any Key Performance Indicators (KPIs), as proposed by the ESHRE Vienna consensus, differed significantly between two of the leading brands of embryo culture media system.

Methods: From June 2017 to December 2017, 467 ICSI cycles were randomized between two embryo culture media systems: Irvine Scientific (Santa Ana, US) or Vitrolife (Gothenburg, Sweden). Randomization corresponded to alternating days over a 7-days/week service. Both groups were controlled so that they did not significantly differ in female patient age, the number of oocytes obtained and rate of oocyte maturity. All microinjected oocytes and embryos were cultured in TLI integrated incubators (EmbryoScope, Vitrolife) to allow detailed KPI analysis. Data was analysed using a 2-tailed Student's T-test and Fisher's exact test ($p < 0.05$)

Results: There was no significant difference between the Irvine and Vitrolife groups in the fertilisation rate, proportion of top quality embryos on Day 3 (35.4% vs. 39.2%) and Day 5 (21.6% vs. 20.0%) and proportion of embryos reaching ≥ 6 cells (Day 3) (78.5% vs 81.2%) and the blastocyst stage (Day 5) (48.9% vs 47.8%). Despite no difference in the Day 5 blastulation rate, there was a significant difference between the Irvine and Vitrolife groups in rate of Day 6 blastocyst development (5.4% vs. 8.3%, respectively, $P < 0.05$). However, the overall embryo utilisation rate (number of embryos transferred and cryopreserved) was not significantly different between the groups.

Discussion: Whilst in vitro culture media impact on the embryo preimplantation development and quality, continuous improvements to both media and culture systems (such as TLI integrated incubators) are making differences negligible. Subtle differences, such as the rate of Day 6 blastocyst development exist, but without full disclosure of culture media constituents, the reason for this remain speculative.

SP1A.5 Can time lapse imaging help identify inner cell mass splitting and reduce the frequency of Monozygotic Twinning after single embryo transfers?

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Background: Despite elective single embryo transfer (eSET) policies, multiple pregnancies may result from monozygotic twinning (MZT). For embryos cultured in vitro, it has been suggested that the method of blastocyst hatching may influence the MZT rate, with '8-shaped hatching' increasing the risk of splitting of the inner cell mass (ICM) compared to 'U-shaped hatching'. For hatching embryos, time-lapse imaging (TLI) allows for detailed scrutiny of whether an ICM may be splitting.

Methods: A retrospective analysis of patients reporting a MZT following eSET was performed over a 4-year period (September 2014–September 2018). MZT was defined as detection of two fetal hearts. Only patients where all embryos were cultured in TLI integrated incubators (EmbryoScope, Vitrolife, Sweden) from the day of fertilisation to the day of transfer (Day 5) were included. Images were analysed to determine the stage of development, ICM grade and if ICM splitting could be observed.

Results: The multiple pregnancy rate following Day 5 eSET was 2.0%(20/1080). 19 blastocysts resulted in MZT and one blastocyst resulted in a MZ triplet pregnancy. 35.0% (7/20) of blastocysts had initiated the hatching process at the time of transfer on Day 5. Of these, only one demonstrated '8-shaped-hatching'. Whilst ICMs were graded as A(9), B(10), C(1) at the time of transfer, upon retrospective analysis, two separate ICMs were observed in 30.0%(6/20) of blastocysts. Splitting of a single ICM was observed on three occasions from hatching embryos.

Conclusions: Multiple pregnancy can occur after eSET if two ICMs form in a blastocyst. This phenomenon can be observed if detailed evaluation of ICM(s) is undertaken to specifically detect if splitting has occurred, and particularly if embryos are hatching. To avoid MZT, blastocysts with two ICMs should be selected against where possible and patients counselled on the risks of MZT pregnancies if an embryo with split ICM is transferred.

SP1A.6 Does the thaw to transfer interval (TTI) affect the outcome of a frozen embryo transfer (FET) cycle?

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Introduction: In 2016 there were over 21,000 FET cycles in UK with a live birth rate of 26% per treatment cycle and 22% per embryo transferred⁽¹⁾. The thaw to transfer interval (TTI) can vary depending on thaw and transfer times, but the effect of the TTI has not been studied broadly and the literature reflects this. However, one study assessed the implantation and live birth rate between long (18-24 hours) and short (2-5 hours) TTI. They showed an inverse correlation between IR and LB rates to the TTI⁽²⁾.

Aims/objectives: This study aims to assess how the TTI (which varies from <2 to >6 hours at our clinic) affects positive pregnancy test rate (POS), clinical pregnancy rate (CP) and live birth rates (LB).

Methods: This retrospective study assessed 735 FET cycles with a single warmed blastocyst and transfer in standard culture media from one clinic between 2015 and 2017.

Results: Overall there were 735 FET cycles with a POS of 51.3%, a CP of 37.7% and a LB of 26.7%. Of the 24 cycles that had a TTI of <2 hours the POS was 54.2%, CP 45.8%, LB 41.7%. 136 cycles had a TTI of 2-4 HRS with a POS of 50.0%, CP 33.8%, LB 25.7%. 541 cycles had a TTI of 4 - 6 HRS with a POS of 51.0%, CP 38.1%, LB 26.4%. 34 cycles had a TTI of >6 HRS with a POS of 58.8%, CP 41.2%, LB 23.5%.

Results: ANOVA testing shows no significant differences in the POS, CP or LB between the groups (p=0.965, 0.934, 0.827). The TTI appears to not affect POS, CP and LB rates, however, a TTI of <2 hours appear to maintain a higher ongoing pregnancy rate compared to longer TTI. More data is required to assess whether this trend is significant.

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Short papers session 1B: BFS early career clinician and scientist prize

SP1B.1 ICSI vs IVF in poor responders: Does the method of fertilization alter the outcome in the absence of a medically indicated reason for choice of insemination? An analysis of 1,071,043 cycles of ART

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Background: Managing patients in whom <4 oocytes are retrieved following controlled ovarian stimulation is one of the most challenging aspects of assisted reproductive treatment (ART). Often, success rates with spontaneous conception and through ART are similar. Selection of the most appropriate insemination method is important to reduce the risk of failed fertilisation and increase the likelihood of a successful outcome. This study aims to determine the optimal method for insemination in poor responders.

Methods: Retrospective cohort analysis of ART cycles between 1991-2012, from the HFEA database. 1,071,043 cycles were identified: 916,132 fresh cycles (IVF 393,956; ICSI 271,219), excluding donor sperm cycles. The singleton live birth rate (SLBR) was stratified by maternal age and stage of transfer, and adjusted by method of insemination. Statistical significance: p<0.05 using Logistic Regression.

Results: Across all age groups, the IVF and ICSI fertilisation rate was 62% and 60%, respectively; $p < 0.0001$. A 7% increase in live birth outcomes (OR:1.067, 95%CI 1.027-1.109, $p = 0.001$), between 1991-2012, is demonstrated for ICSI cycles compared to IVF. However, sub-analysis of cycles between 2002-2012 demonstrates a 9% increase in live birth outcomes with IVF (OR:1.087, 95%CI 1.052-1.122, $p < 0.0001$). The increase is maintained when controlled for the cause of subfertility, with a 14% increase in live birth rate (OR:1.139, 95%CI 1.095-1.186, $p < 0.0001$) with male factor as the reference category. A blastocyst transfer resulted in an 84% increase in live birth when compared to cleavage stage (OR1.842, 95%CI 1.714-1.979, $p < 0.0001$). Overall, the live birth rate increased by 37% (OR:0.389- 0.763) as the number of eggs collected increased from 1 to 3; $p < 0.0001$.

Conclusions: ICSI initially demonstrated a benefit over conventional insemination when < 4 oocytes are retrieved, with a 7% increase in the live birth rate, however, further analysis of more recent cycles, accounting for advancements in technique, supports IVF in cases without evidence of concomitant male factor subfertility

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SP1B.2 Going, gonad, gone: A comparison of the management of gonadal torsion between men and women

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Introduction: Gonadal torsion is a surgical emergency in both men and women causing vascular constriction and ultimately necrosis of testicular or ovarian tissue. Prompt management is essential to preserve gonadal function.

Aims: To compare the timeliness with which men and women with suspected gonadal torsion are managed and the effect this has on the fate of their gonads.

Methods: We retrospectively reviewed all adult patients who underwent surgery for suspected gonadal torsion between 1/4/16 and 31/3/18. We recorded the following times: symptom onset; hospital presentation; and knife-to-skin (KTS). We also documented the surgical procedure(s) and intra-operative findings.

Results: 31 women (mean age 29.4+/-7.1yrs) and 49 men (mean age 23.2+/-7.0yrs) underwent surgery for suspected torsion. The median time from symptom onset to hospital presentation was 3.3hrs (IQR:1.4-3.9hrs) in men and 21.6hrs (IQR 7.9-78.1hrs) in women ($p < 0.00001$) and from hospital presentation to KTS was 4.0hrs (IQR 3.1-5.4hrs) in men and 26.2hrs (IQR:12.3-47.5hrs) in women ($p < 0.00001$). The median time from symptom onset to KTS was 7.1hrs (IQR:5.4-14.3hrs) in men and 74.3hrs (IQR:39.0-100.3hrs) in women ($p < 0.00001$). Surgery occurred within six hours of symptom onset in 19 (38.8%) men but no women and more than 48 hours later in 21 (67.7%) women and only 5 (10.2%) men. Intraoperatively, torsion was confirmed in 20 (64.5%) women and 25 (51.0%) men but 13 (65%) ovaries were necrotic compared to only 6 (24%) testes ($p = 0.0076$). All necrotic gonads were removed.

Conclusion: Notwithstanding the fact that women delay seeking help, once in hospital they wait, on average, six times longer than men for surgery for suspected gonadal torsion. When suspected, the incidence of actual torsion in both genders is high, but, due to various (often avoidable) delays in women, the majority of ovaries are necrotic compared with less than a quarter of testes. Women with suspected gonadal torsion receive suboptimal care compared to their male counterparts, which has potentially catastrophic consequences for their fertility.

SP1B.3 The cytotoxic impact of repeated cisplatin exposure in vitro on markers of tissue health in ovine ovarian cortex

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A widely reported side effect of chemotherapy treatment is ovarian failure. The exact mechanism is unclear and extent of damage is difficult to predict *in-vivo*. We report an *in-vitro* ovine model of the cytotoxic impact of cisplatin on ovarian cortex during single (Experiment 1) and repeat exposures (Experiment 2).

Experiment 1: Ovarian cortex was harvested from abattoir-derived sheep ovaries. 3mm diameter biopsies were cultured for 72hr in defined serum-free medium with exposure to 0-100µg/ml of cisplatin from day 2-3. Spent media was assayed for LDH release and tissue was snap-frozen for future mass spectrometry analysis of cisplatin content. LDH concentrations were expressed in IU/mg of tissue. No differences in LDH concentration were detected prior to exposure. Media LDH content was significantly higher following tissue dissection than cisplatin exposure across all treatments ($p \leq 0.0001$). LDH activity was

reduced in tissue exposed to 4µg/ml (0.00036±0.0001U/mg), 7µg/ml (0.00030±0.00008U/mg) and 100µg/ml (0.00029±0.00006U/mg) cisplatin compared to unexposed controls (0.00075±0.0001U/ml, n=11, p=0.005).

Experiment 2: Tissue and follicle viability were assessed by 180min incubation in 15µg/ml of the vital dye neutral red on Day-0 and Day-22 of culture. Initial and end weights were recorded. 2-way ANOVA with post hoc Tukey's test were used for analysis. Data was collected across 8 replicate cultures, n=10 biopsies/treatment. Tissue and follicle viability were significantly reduced with increasing cisplatin concentration (p=0.0002) and repeat exposure (p<0.0001). Tissue weight decreased by 30±1.7% from 4.5±0.2mg to 3.1±0.1mg during culture across all treatments (n=168, p<0.0001), this was consistent across treatments and exposures (p=0.4). Media LDH concentration was unaffected by cisplatin dose or number of exposures (p=0.8), but decreased throughout culture (p<0.0001).

Tissue and follicle damage can be rapidly quantified following repeat exposures to cisplatin in this model. Further studies will quantify the long-term accumulation of cisplatin within tissues and the effects on cortex metabolism.

SP1B.4 Direct effects of androgen on genes regulating cholesterol biosynthesis in human granulosa-lutein cells

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Polycystic ovary syndrome (PCOS) is a common endocrinopathy, affecting 5-10% of women of reproductive age. It is a complex heterogeneous disorder of ovarian function characterised by hyperandrogenism, menstrual disturbance and polycystic ovarian morphology^[1]. Ovarian steroidogenesis and hence androgen production rely upon cholesterol as a substrate. Previous whole genome RNA-Sequencing in our lab has identified 21 genes involved in cholesterol biosynthesis that are downregulated in the granulosa-lutein (GL) cells of women with PCOS^[2].

To test the hypothesis that the observed differences in cholesterol biosynthetic gene expression are due to direct effects of excess androgen, we treated GL cells with dihydrotestosterone (DHT). Using RT-qPCR we investigated the effects of in vitro androgen treatment on the expression of 20 cholesterol biosynthesis genes. Treatment of GL cells in primary culture with 10 nM DHT resulted in significant downregulation of the key cholesterol biosynthetic genes *MVD*, *IDI1* and *FDPS* (P<0.05).

Preliminary protein work using immunohistochemistry in adult mouse ovaries localised two key cholesterol biosynthesis enzymes (HMGCS2, DHCR7) to the granulosa cells (GC) of developing follicles and demonstrated that GC specific expression was inversely correlated with follicle size. Highest expression of the two enzymes was in corpora lutea (CL) which accords with the high steroidogenic capacity of the CL.

These results suggest that the downregulation of cholesterol biosynthesis genes seen in PCOS may in part be due to hyperandrogenism. We therefore propose a mechanism whereby androgens downregulate cholesterol biosynthesis genes as a homeostatic mechanism to compensate for the increased rate of steroidogenesis seen in granulosa cells of women with PCOS.

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SP1B.5 Dysregulation of the Interleukin-17 pathway in women with unexplained infertility affects pregnancy outcome following assisted reproductive therapy

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Background: Implantation failure is being increasingly recognised as a critical factor in unexplained infertility and may be an increasingly important component of success in ART. The objective of this study was to utilise mid-luteal endometrial scratch biopsies to identify gene expression patterns, and pathways associated with successful implantation in women with unexplained infertility using RNA-sequencing.

Methods: Using a prospective longitudinal study design, nulliparous women with unexplained infertility undergoing ART were recruited, following strict inclusion criteria. Endometrium and matched serum samples were accurately timed and taken at the mid-luteal stage of the menstrual cycle. Patients underwent a single embryo transfer in the subsequent cycle. Next generation RNA-sequencing was performed using NextSeq550 and analysed using EdgeR. Validation was performed on a selection of genes using TaqMan quantitative real-time PCR (qRT-PCR) and an ELISA for serum and tissue.

Results: 29 women met inclusion criteria; 20 'gene discovery cohort' and 9 'independent validation cohort'. RNA-sequencing was performed on samples from the discovery set; nine (45.0%) had a successful pregnancy and eleven (55.0%) did not. There were no differences in baseline characteristics. 204 protein-coding genes were differentially expressed (DEG) between

the groups ($p < 0.05$); 168 genes with decreased and 38 with increased expression in women with successful pregnancy. Over-represented pathways included 'Interleukin-17 (IL-17) signalling pathway' (6 genes; $p = 0.001$), 'PI3K-Akt signalling pathway' (12 genes, $p = 0.001$) and 'Cytokine-cytokine receptor interaction' (10 genes, $p = 0.002$). All DEG in the IL-17 signalling pathway, MMP3, MMP1, IL1 β , LCN2, S100A9 and FOSL1 had decreased expression in the pregnant group. These findings were confirmed using qRT-PCR and an ELISA demonstrated increased IL-17 in serum and tissue in both cohorts.

Conclusions: IL-17 is a pro-inflammatory cytokine that plays a key part in inflammation and autoimmunity. It has been shown in the pathogenesis of several immunoinflammatory diseases and biologic agents have been developed targeting this pathway. Our novel findings have the potential to lead to the development of diagnostic and therapeutic strategies to improve pregnancy outcomes in women with unexplained infertility.

SP1B.6 Monitoring follicle number and serum oestradiol during ovarian stimulation may provide false reassurance in GnRH-antagonist cycles in women at high risk of OHSS

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Introduction: In modern practice, ovarian reserve tests are used to identify women at high risk of OHSS, allowing use of lower-risk stimulation techniques in this group. Despite this, the incidence of OHSS has not declined over several years (NHS HES data). We asked the question - is this due to failure of screening tests to identify high-risk women or due to decisions made during ovarian stimulation?

Setting: Tertiary hospital with affiliated NHS clinic performing approximately 1100 stimulated IVF cycles annually.

Methods: We prospectively identified cases of moderate or severe OHSS in antagonist cycles between January 2017 and June 2018. Medical records were reviewed by AS and RM to confirm diagnosis and classify severity (RCOG classification). Data were extracted from IVF notes by AS and SB for ovarian reserve, ovarian response, stimulation regime, trigger, elective cryopreservation and cycle outcome.

Results: Twenty-four cases of OHSS (11 severe and 13 moderate) were identified (incidence 2%). Median AMH was 56.58 pmol/l (range 18.3-203) in severe and 31.98 pmol/l (range 9.28-147) in moderate cases, median AFC was 20 (10-41) in both groups. 9/11 severe OHSS and 9/13 moderate OHSS occurred in women with high AMH (> 35 pmol/l) and/or high AFC (> 20). Ovarian response was excessive ($E2 > 15000$ pmol/l and/or > 20 follicles > 14 mm) in only 4/11 severe and 4/13 moderate OHSS cases. Hence, 16/24 (67%) of significant OHSS cases occurred in cycles where response to stimulation was not excessive.

Discussion: Our analysis suggests that monitoring ovarian response during stimulation may provide false reassurance in women with high ovarian reserve undergoing GnRH-antagonist cycles. We propose that GnRH-agonist trigger and elective cryopreservation should be considered in this group, even if the response to stimulation is not excessive. This requires prospective evaluation. Reassuringly, ovarian reserve tests predict the majority (76%) cases of significant OHSS in antagonist cycles.

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Short papers session 1C: Enhancing our practice through education and patient expectations

SP1C.1 Fertility nursing education and career progression framework

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Fertility nursing is a specialist area of practice where nurses are at the forefront of an emerging care setting, do we need to provide an educational framework to support this? Fertility nursing encompasses the care and practices undertaken by any registered nurse/midwife providing fertility care, an educational framework is paramount for those individuals. Fertility nurses have increased levels of responsibility. Political drivers, workforce requirements and nursing care require multidisciplinary collaboration; whilst it is recognised that not every nurse/midwife will aspire to Masters' level advanced practice, all are expected to reach the maximum potential expected of their role in the context of competence and knowledge. Equally the need for nurses to become specialists in their particular field of practice, to enhance overall service

provision, through clinical leadership and be recognised as expert practitioners includes understanding the socio economic and political dimensions to delivering care, and ensuring service meets the needs of those seeking fertility care.

A career progression and education framework has been constructed by senior fertility nurses, professional stakeholders and peers. The framework has been published and shared with professional stakeholders for review. The framework is a publication and booklet for fertility nurses and Health care assistants throughout the UK and contains tools for self-assessment for competency. The project was the focus of the group for 2017 and has taken a total of 5 months to complete.

The project group consisted of 12 core contributors, 3 external advisers and a wider group of stakeholders. The work was mainly constructed by individuals of the core group and forwarded to the group chair for amalgamation and proofing. Teleconferences and face to face meetings took place to discuss project content and for any amendments to be made. Each member of the core group were responsible for separate parts.

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SP1C.2 Meeting the learning needs of Health Care Assistants within a fertility setting

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Health Care Assistants form a vital part of the multi-disciplinary team working alongside registered nurses, they provide the hands-on care for fertility patients and have an important role to play in their journey and experience. There are educational days available for qualified nurses in the fertility sector however an analysis by SING (Senior Infertility Nurse Group) in 2016 showed a lack of training and educational support available for HCAs in the fertility setting. The findings from the data initiated the decision to organise a study day with an agenda specific to fertility HCAs. The content for the day was designed by representatives from SING. The initial pilot study day was held in 2017, 36 HCAs attended representing fertility units across the UK and Ireland. The course content included taking fertility treatment back to basics, followed by group work and roundtable discussions.

36 feedback forms were completed and analysed. The following methodology was used; on a scale of 1 (poor) to 4 (very good) the HCAs provided (by multiple choice) their feedback in relation to the following areas: programme design, length,

workload, method of instruction and delegate involvement. Forms were anonymised to ensure both positive and negative feedback could be shared.

Other findings mentioned how the group felt vulnerable and often undervalued in their setting. Further comments both verbally and written were made explaining that after this particular study day they felt empowered, valued and proud to be an HCA.

It should be the responsibility of the fertility service to provide support for HCAs in order to develop their knowledge and skills and enable them to provide the excellent standard of care expected. This study day has made a step towards filling this gap with a second study day being held in 2018.

SP1C.3 Complexities of managing a donor sperm programme in 2018

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Jessops Fertility, UK

Background: The legal parenthood consent process for donor sperm recipients is complex and, in recent years, errors have led to distressing and much publicised court cases. Centres have to ensure there is an effective system in place to eliminate errors and ensure patients have all the correct, essential information to make informed decisions about legal parenthood status for any potential child. The correct consent must be in place prior to any treatment can go ahead. Over the last few years patients and their social situation have become more complex, presenting with many different scenarios to manage. The role of an experience clinical specialist (CNS) is there pivotal.

Method: Jessops fertility has a dedicated CNS to manage all patients requiring donor sperm. Complex cases such as patients who are separated but not divorced from their previous partner, these wishing to proceed with inter partner oocyte donation, those wishing to have sibling pregnancies after they have separated as a couple, follow a pathway managed by the CNS to minimise errors. Complex case meetings held jointly with the person responsible ensure that the legal and regulatory requirements are adhered to. Mapping of the pathway ensures that checks of consents are in place at critical times in the patient cycles. An annual audit matrix is undertaken to ensure all patients having donor sperm have all aspects of their treatment, including all consents, comprehensively audited.

Results: Results from audits have shown that in 2017 there was full compliance with the regulatory and legal requirements as set out in the HFEAs Code of Practice. All 67 patients who were treated with donor sperm had marriage status checks present in the notes, had attended counselling and had completed all consents prior to treatment. Having a dedicated CNS to manage the donor programme has minimised errors and improved the pathway for patients.

SP1C.4 Managing a patient's expectations of miscarriage following assisted conception

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Purpose: To confirm if a CRL measurement taken between 6-8 weeks gestation measuring behind gestational age predicts miscarriage, enabling appropriate counselling to couples following assisted conception. Furthermore, if a patient opts for medical management of miscarriage following an assisted conception is the resolution outcome the same as for natural conceptions.

Methods: A retrospective review of patients between March 2016 - November 2017 with confirmed miscarriage undergoing medical management of miscarriage with a case-matched control group of women who achieved a live birth following assisted conception.

Results: 50 patients had a confirmed miscarriage and opted for medical management; of these, 20 women had a fetal heart beat before confirmed miscarriage. The average days difference in gestational age compared with an age and treatment matched control group who achieved a live birth was 7.35 vs 1.93 (n=60; p<0.0001). A CRL measurement >2 days different to gestational age had an OR 25.71 (95% CI 3.13-211.26; p=0.0025) for eventual miscarriage. These differences were similar between fresh or frozen treatment cycles. 28% of the women required further intervention for miscarriage with either repeat medical management or surgical evacuation of the uterus following one dose of 800mcg misoprostol. Mean time from initiation of medical management to confirmation of successful miscarriage treatment was 31.6 days (range 11-103) including repeat procedures.

Conclusions: The results of a pregnancy scan following fertility treatment, if not appropriate for gestational age, may enable the clinician to counsel the patient about the anticipated outcome. Of those who opt to have medical management, one third of women require a second medical or surgical treatment using our current single dose misoprostol protocol. Resolution of miscarriage on average takes 31 days. These figures are in keeping with published literature for naturally conceived pregnancies, highlighting no difference in managing assisted conception pregnancies that miscarry.

SP1C.5 2018 Study of 485 adult donor-conceived people

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Donor Sibling Registry, USA

Objective: The purpose of the study was to better understand donor-conceived adults and their individual differences with regards to: viewing their own donor conception, their desire to search for the donor, search methodologies used, and if the donor was found, how they defined that relationship.

Design: Online questionnaire for adult donor offspring with qualitative and quantitative questions.

Materials and method: The online survey was made available to Donor Sibling Registry members for a 3 week period from 12/17-1/18.

Results: Nineteen (3.9%) of respondents were conceived with an egg donor and 466 (96%) with the help of a sperm donor. 76.5% were female, 20.3% male. The age range was from 18-74 with 28.76 being the mean age of participants. LGBT parents raised 27% of the participants.

Disclosure: 85.1% were told that they were donor-conceived by their parents. 3.3% said that someone else told them, and 11% reported that they found out "on their own".

Searching for their donor: 70.3% had tried to locate/find their donor, while 29.6% indicated that they had not done so.

Methods for used for locating/finding donors: (participants could select all that applied): 17.6% used a record search, 24.1% utilized DNA testing, 21.2% contacted the fertility clinic/doctor/sperm bank, 31.6% used the DSR, .6% hired a genealogist and 4.9% used other means of searching.

Contacting donors: 30.9% participants said that they knew the identity of their donor and 66.6% of them had attempted contact. 16.7% through social media (e.g. Facebook), 11.8% via DNA testing, 11.3% through the DSR, 26.7% sent a personal letter or email, 11.8% called or texted the donor, 15.8% reached out to the donor's family, and 5.9% used other means to contact the donor. 84% agreed with the statement, "I think a lot about the characteristics I might share with my donor." 60.7% agreed, "I understand myself better because I have thought about who I am in relation to my parents and donor." 42.9% felt it was important to be in contact with other donor-conceived individuals.

SP1C.6 Understanding attitudes towards female genital mutilation and cutting among women (FGMC) attending antenatal clinic

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Introduction: Women's equality and empowerment is one of the UN Sustainable Development Goals and female genital mutilation/cutting (FGMC) is a violation of the basic rights of women and girls. Health implications include shock, haemorrhage, sepsis, sexual dysfunction and death. FGMC is usually perpetuated without the victim's consent or awareness of possible health implications. This study therefore aimed to evaluate the knowledge, attitudes and practices of FGMC among women attending antenatal clinic.

Methods: Study participants for this cross sectional study were selected using systematic sampling. Information was obtained from 225 respondents using semi-structured, interviewer administered paper-questionnaires over a 3-month period from June to September 2017. The data was analysed using SPSS version 20.

Results: Majority (79.1%) of the respondents had primary school as their highest level of education. Most of the respondents in this study (93.3%) were aware of female genital mutilation/cutting. However, only 16.9% had knowledge of the health implications of the practice. There was a statistically significant relationship between knowledge and level of education. Nearly half of the respondents 42.9% had poor attitudes towards female genital mutilation/cutting. There was a statistically significant association between the educational status of the respondents and desire to circumcise their female children ($p=0.005$). Nearly one third of the respondents had experienced some form of female genital mutilation/cutting before puberty. Over one-fifth of respondents (20.4%) plan to continue the practice on their daughters.

Conclusions: The knowledge of the health implications of FGMC among respondents is poor and majority viewed FGMC in a favourable light. Lower levels of education are associated with increased practice of FGMC. Improved education and awareness campaigns on the dangers of this practice are needed to stop this violation of the basic rights girls and to end all forms of violence against women.

Short papers session 1D: SRF PhD student prize

SP1D.1 Exosomes for mammalian sperm cells: Assessing the impact of this novel delivery tool for future clinical applications

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Purpose: Male infertility accounts for 35-50% of infertile cases^[1]. Several approaches towards treating sperm-borne aberrations are currently being explored. Exosomes are extracellular vesicles released by all human cell-types, with a non-immunogenic nature and proven cargo-delivery that rival with synthetic-based systems for compound delivery with reproductive purposes^[2]. However, safety aspects have not been extensively investigated for their use with gametes. In this study we isolated and characterized exosomes, and assessed their *in vitro* exposure-effects in boar sperm. More precisely, viability, mitochondrial membrane potential (MMP) and membrane fluidity (MF) were evaluated in *in vitro* capacitated and hyperactivated-induced spermatozoa.

Methods: Exosomes (EXO) were synthesised from Human Embryonic Kidney (HEK)293T cells, and characterised by Nanoparticle Tracking Analysis (NTA), Western Blotting and Transmission Electron Microscopy (TEM). Boar sperm (n = 5) was *in vitro* co-incubated with increasing concentrations of EXO (10⁶, 10⁷ and 10⁸) under capacitating and progesterone-induced hyper-activating conditions. Aliquots were retrieved at 0h, 2h, 4h, 4h10, 4h30 and 5h and stained for flow cytometry; viability (SYBR-14/Propidium iodide), MMP (JC-1) and MF (YO-PRO-1/Merocyanine-540). Data were analysed with a mixed model (between-subjects factor: treatment; within-subjects factor: incubation time) followed by post-HOC sidak test for pair-wise comparisons.

Results: TEM, WB and NTA showed exosome-like morphology, Alix, Syntenin and CD9, and a size and concentration profile of 158nm and 1.23x10¹⁰ EVs/mL, respectively, in EXO samples. Flow-cytometric analyses resulted in no significantly different viability, MMP and MF in any of the ratios at any time point when comparing them with the controls.

Conclusions: An efficient exosome-enrichment was achieved with optimised protocols easily reproducible towards future clinical applications. Additionally, high concentrations of exosomes did not compromise the viability nor the response of boar spermatozoa to induced capacitation and acrosome-exocytosis *in vitro*. These encouraging results shed light on the potential behind exosomes for their future clinical applications in reproduction.

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SP1D.2 Mechanism of egg activation during fertilization: Identifying sperm & egg factors, their role in egg activation and development

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Background: Calcium oscillations are essential for activation of the egg and a series of post-fertilisation events. Phospholipase C zeta (PLCζ) knockout greatly inhibits the majority of the sperm-derived Ca²⁺ oscillations confirming PLCζ's physiological importance^[1]. However, surprisingly, a few delayed Ca²⁺ oscillations still occur in the absence of PLCζ, and these result in an unexpected subfertility, not infertility, in such mice^[2]. This suggests that PLCζ is not the sole factor to initiate Ca²⁺ oscillations in the egg and that other sperm or/and egg-derived factors are involved. This encouraged us to explore other sperm/egg derived PLC isoforms & their potential roles in egg activation.

Methods & results: Qualitative and quantitative genetic and protein expression of PLC isoforms suggest many of the mammalian PLCs are expressed in both sperm and eggs. qPCR identified significant changes in expression of certain PLCs in PLCζ KO sperm cells which make them target PLCs to study in the context of fertilisation. Our pharmacological analysis has also suggested potential roles for other PLCs in the activation of eggs in the absence of PLCζ. Preliminary studies on WT and PLCζ KO blastocyst development suggests that the differences in the Ca²⁺ signal after fertilization in PLCζ's absence has an important impact on embryo development.

Conclusion: This suggests that other PLCs besides PLCζ may have an important role in the egg activation process. Ca²⁺ transients and egg activation that occur in PLCζ's absence after fertilization could be due to such PLCs. Inhibiting sperm and egg PLCs separately has an effect on the Ca²⁺ signals induced at fertilization with WT as well as PLCζ KO sperm.

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Ca²⁺ oscillations that induce embryogenesis in mammals but conception can occur in its absence. *Development* (Cambridge, England). 2017;144(16):2914-2924.

SP1D.3 Gonadal tissue concentrations of phthalates perturb early follicle development in mouse ovarian explant cultures

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Introduction: A commonly used plasticiser, Di-2-Ethylhexyl Phthalate (DEHP), leaches from many plastic products and is ubiquitous in the environment. DEHP, and its primary metabolite Mono-2-Ethylhexyl Phthalate (MEHP), have endocrine disrupting activity and adversely affect ovarian function. We hypothesised that such effects may be a sequelae of exposure-linked perturbations of early ovarian follicle development.

Methods: As an index of human ovarian phthalate contamination, DEHP was measured in 16 dog ovaries: a recognised sentinel species. Murine ovaries were exposed to 0.6x, 6x and 60x mean wet weight (µg/ml) of dog ovarian DEHP (measured) and MEHP (estimated) concentrations, using two different culture methods. (1) Whole Post Natal Day (PND) 4 ovaries exposed to phthalate for 6 days and (2) fragmented PND 5 ovaries cultured for 5 days and monitored real-time during a 24h exposure. Bouins fixed ovary sections were examined for BrdU (proliferation marker), added to culture 24h pre fixation, by immunohistochemistry. Real-time effects on relative follicle health before and after exposure was assessed by trypan blue exclusion (oocytes/granulosa cells stained).

Results: In whole ovary cultures, primordial follicles as a proportion of total follicles were increased by DEHP [60x: P<0.05] and decreased by MEHP [60x: P<0.05]. The proportion of follicles that were unhealthy was significantly increased for primordial, transitional and total follicles by DEHP (60x) and MEHP (60x) [P<0.05]. Additionally, MEHP (60x) increased primary follicles while decreasing secondary follicles [P<0.05]. A significant increase in follicular proliferation was observed [P<0.05] at 6x DEHP as well as at 6 and 60x MEHP [concentration effect: P<0.05]. In fragmented ovarian culture, DEHP and MEHP increased the proportion of follicles that were unhealthy at all levels of exposure [P<0.05: all effects; n=24].

Conclusion: Exposure to either DEHP or MEHP perturbs follicular proliferation and decreases follicle health. This may be a presage of phthalate associated disturbances in ovarian function.

SP1D.4 Rapamycin reduces the adverse impact of PTEN inhibition on DNA damage response without preventing increased follicle growth activation

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Objectives: Can mTOR inhibition modify the increased oocyte DNA damage from PTEN inhibition without preventing increased follicle growth activation?

Methods: Bovine ovarian fragments (4x2x1mm) were exposed to medium containing 1) 1µM PTEN inhibitor bpv(HOpic), 2) 1µM bpv(HOpic)+0.1nM rapamycin, or 3) control medium for 24 hours. Tissue was incubated for a further 5 days in control medium then fixed and analysed histologically by immunostaining for γH2AX, a marker of DNA damage, and the DNA repair proteins MRE11, ATM, Rad51, BRCA1 and BRCA2.

Results: 12,242 follicles were analysed. Tissue exposed to 1µM bpv(HOpic) contained a significantly higher proportion of growing follicles (81.9%) compared to 1µM bpv(HOpic)+0.1nM rapamycin (70.2%) (p=0.01) and control (50.9%) (p<0.001). A significant increase of growing follicles was observed in 1µM bpv(HOpic)+0.1nM rapamycin (70.2%) compared to control (50.9%) (p<0.001). Expression of γH2AX in oocytes in primary follicles was highest in 1µM bpv(HOpic) (64.5%) compared to 1µM bpv(HOpic)+0.1nM rapamycin (29.5%) (p<0.001) and control (44.4%) (p=0.044), no changes were observed between control and 1µM bpv(HOpic)+0.1nM rapamycin group (p=0.119). Expression of MRE11, ATM and Rad51 was reduced in oocytes of primary follicles exposed to bpv(HOpic) (69.5%, 30.1% and 31.3% respectively) compared to 1µM bpv(HOpic)+0.1nM rapamycin (85.7%, p=0.033; 65.3%, p<0.001; 49.3%, p=0.007) and control (97.5%, p<0.001; 65.0%, p=0.014; and 90.6%, p<0.001). No differences in MRE11 and ATM expression were found between 1µM bpv(HOpic)+0.1nM rapamycin treatment and control, but Rad51 was significantly lower in the combined treatment (p=0.001). Neither bpv(HOpic) or combined bpv(HOpic)+rapamycin affected BRCA1 expression in the oocytes of primary follicles (13.5 and 8.0% respectively) compared to control (5.7%) (p=0.667 and 0.264). bpv(HOpic) increased BRCA2 expression (63.0%) compared to control (49.0%) (p=0.045) but BRCA2 expression in the combined treatment was not different from controls (56.0%) (p>0.05).

Conclusion: Addition of a low dose of rapamycin to bpv(HOpic) reduced DNA damage and improved DNA repair of oocytes whilst maintaining increased follicle activation.

SP1D.5 Mouse embryos are susceptible to cohesion fatigue

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Background: Preimplantation embryos often comprise cells with the correct (euploid) and wrong number of chromosomes (aneuploid) due to chromosome segregation errors. Such 'mosaic' embryos may reduce reproductive success, but how these errors arise remains unclear. The timing of early embryo cell divisions is highly variable in embryos, and is emerging as a potential indicator of embryo health in clinics (morphokinetics). In somatic cells, extended mitosis can cause premature separation of sister chromatids, a phenomenon known as 'cohesion fatigue', which could cause aneuploidy. Here, therefore, we set out to determine whether embryos are susceptible to cohesion fatigue.

Methods: We manipulated the duration of mitosis in two-cell stage embryos with the APC inhibitor APCin. Live 3D confocal imaging of Sir-Tubulin, H2B:RFP and MajSatTALE:mClover to monitor spindle, chromatin, and pericentromeric regions, respectively, revealed that mitotic arrest triggered a pronounced spindle elongation and loss of chromosome alignment. Fixed cell experiments confirmed a time dependent loss of chromosome alignment, and revealed that almost all of the misaligned chromosomes (95%) were prematurely separated sister chromatids, as opposed to sister-pars.

Results: The loss of sister cohesion was preceded by an increase in inter-kinetochore distances from 0.59µm to 0.79µm ($p < 0.0001$), consistent with cohesion fatigue. Formal counting of the proportion of separated sisters by labelling kinetochores after spindle disassembly revealed that 4% of all sister pairs had individualised by 6 hours of mitotic arrest, and 66% by 24 hours. Strikingly, live cell and fixed experiments suggest that the earliest cohesion fatigue events can happen as little as 2 hours into mitosis, a timescale not dissimilar from the normal duration of mitosis. We therefore speculate that cohesion fatigue may be a previously unappreciated cause of aneuploidy in the preimplantation embryo.

SP1D.6 The presence of trophoblast spheroids alters Toll-like receptor 5 activation in endometrial cells in an in vitro model

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Background: Extracellular vesicles (EVs) mediated communication between the female reproductive tract and the pre-implantation embryo seems to play an essential role in the successful establishment of pregnancy (Ng, 2013). In addition, it is well documented that, Toll-like receptors (TLRs) activation will influence trophoblast-endometrial interactions (Sanchez-Lopez, 2014). TLR5 is responsible for the recognition of bacterial flagellin, initiating a signalling cascade that results in the production of proinflammatory cytokines, including interleukin (IL)-1β; and IL-6 (Honko, 2005). This study investigated how IL-1β; and IL-6 expression in endometrial RL95-2 cells in co-culture with human choriocarcinoma (JAR) spheroids, alters in the presence/absence of flagellin. In addition, we also investigated the characteristics of EVs secretion in RL95-JAR spheroid co-cultures.

Methods: A two-dimensional culture system was used to study human trophoblast-endometrial interaction prior to implantation. RL95-2 cells were co-cultured with JAR spheroids, separated by an insert, in DMEM/F12 EV-depleted media for 24 hours. Subsequently, inserts were removed and RL95-2 cells were stimulated with 100ng/ml of the TLR5 specific ligand flagellin, for 8 hours. RL95-2 RNA was extracted, reverse transcribed, and qPCR was performed. EVs were isolated from conditioned media using size exclusion chromatography and EV size, concentration and zeta potential evaluated by nanoparticle tracking analysis.

Results: When exposed to flagellin, RL95-2 cells in co-culture with JAR spheroids showed significantly higher IL-1β; and IL-6 expression compared to RL95-2 cells cultured alone ($p < 0.05$). The size and concentration of EVs in conditioned media of RL95-JAR spheroid co-cultures were significantly different to EVs secreted by RL95-2 cells alone ($p < 0.05$). Conclusion: These findings indicate that in the endometrial epithelium, stimulation of the TLR5-mediated innate immune response by bacterial infection may be more pronounced in the presence of the embryo. In addition, bacterial flagellin may influence EV-mediated endometrial-trophoblast communication, indicating a possible diagnostic tool for infection-induced embryo implantation failure.

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SRF Post-Doctoral prize

SRF.1 Last-minute K-fibre establishment underpins age-related chromosome segregation errors in mouse oocytes

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Chromosome segregation errors that arise during the first meiotic division (MI) lead to the formation of aneuploid eggs, the incidence of which increases with age and is considered a major contributing factor of age-related decline in female fertility. Accurate chromosome segregation requires timely formation of stable end-on attachments between kinetochores and microtubules (K-fibres) prior to anaphase. Here, we examined temporal dynamics of K-fibre formation in oocytes from young and old mice.

Live imaging of centromeres and DNA in young and old oocytes during MI revealed retarded chromosome alignment, and increased frequency of lagging chromosome in old (58.8%) versus young oocytes (26.7%), alluding to erroneous K-fibre formation in old oocytes. We found that in both young and old oocytes, the proportion of stable end-on MT-kinetochore attachments gradually increased over time at the expense of lateral attachments to reach the maximum level at the time-point just prior to the anaphase I. The proportion of merotelic misattachments, although initially higher in old oocytes, eventually decreased to the level equivalent to that of young oocytes as the MI progressed, suggesting that the error correction mechanisms do operate in old oocytes. Strikingly, the proportion of chromosomes lacking stable MT attachments gradually decreased over time in young oocytes and became negligible at the time-point prior to anaphase, but remained constantly high (20-30%) throughout entire MI in old oocytes. However, at the onset of anaphase, all chromosomes in both young and old oocytes become stably attached, suggesting that a large proportion of chromosomes in old oocytes rapidly stabilize their attachments just prior to anaphase. Consistently, lagging chromosomes, which are indicative of segregation error, possessed abnormal MT attachment statuses.

We propose that the sudden formation of stable kinetochore-microtubule attachment might promote incorrect attachments and could explain the increased incidence of chromosome segregation errors found in old oocytes.

SRF.2 Spontaneous failure of mouse oocytes to reach metaphase II during maturation is overcome by inhibiting the spindle assembly checkpoint protein Mps1

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Background: Oocytes have a prolonged progression through the first meiotic division (MI), followed by immediate entry into meiosis II and a metaphase (metII) arrest. These cell cycle transitions are controlled by cellular checkpoints whose regulation are not fully resolved. One important control mechanism is the Spindle Assembly Checkpoint (SAC) which can cause an arrest during MI in response to DNA damage^[1,2]. The Mps1 inhibitor reversine, can overcome this arrest in oocytes subjected to DNA damaging agents^[1]. However, during unperturbed maturation some oocytes become spontaneously arrested and if these oocytes respond to reversine is unknown.

Methods: Oocytes were collected from PMSG-primed female MF1 and C57Bl/6 mice and in vitro matured following release from milrinone. MI arrested oocytes, assessed at 12h after milrinone washout, were treated with 1M reversine for 2h. Eggs were activated using strontium-containing media. All time lapse imaging and cell culture was carried out using Biostation IMQ (Nikon,UK).

Results: Oocytes that spontaneously MI arrested (n=40) progressed to metII following reversine (i.e. at 14h, 88%, 36/40); assessed by the extrusion of a polar body. Hoechst staining revealed these oocytes had matured to metII. Vehicle-treated oocytes in contrast remained at MI. These reversine-generated eggs could be activated in response to strontium as was confirmed by visible pronuclei and capacity to form 2-cell parthenotes.

Conclusions: Spontaneously arrested MI oocytes are arrested in their maturation as a result of SAC activation because it is overcome by treatment with the Mps1 inhibitor reversine. We currently do not know why oocytes spontaneously arrest, this maybe because of previous DNA damage, but the present data suggest these oocytes are competent to form viable eggs.

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SRF.3 Expression of the secretory leukocyte protease inhibitor (SLPI) in bovine ovarian tissue and its potential role in cell migration associated with follicle and corpus luteum (CL) 'turnover'

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Introduction: SLPI is a cationic protein and a member of innate immunity associated proteins. There is no information about this protein in bovine; however there are some studies on humans. This protein is considered as an anti-elastase because it has been isolated from secretions of patients with pulmonary disease and cystic fibrosis¹. Cyclic ovarian function involves extensive tissue remodelling and the CL grows faster than any known tumour. Ovulation has been likened to an inflammatory response and TNF- α induces SLPI expression by cultured granulosa cells (GC) ². Here we investigated (1) SLPI expression in GC and TC of developing follicles and CL; (2) the effect of SLPI knockdown on stromal and thecal cell migration.

Methods: RNA was extracted for RT-qPCR analysis of gene expression in GC and TC layers from bovine follicles (1-18mm) and early, mid- and late-stage CL. For in vitro cell migration assays bovine TC and cortical stromal cells (SC) were cultured (10% serum) for 2 days RNA interference was used to examine effects of SLPI knockdown on cell migration and gene expression. A 'scratch' was made in the near-confluent monolayer and % wound closure assessed over 24h. Also gene expression analysed by RT-qPCR.

Results and discussion: SLPI expression increased with follicle size in both GC and TC ($p < 0.0001$). There was also high expression of SLPI in CL. Knockdown of endogenous SLPI expression in both TC and SC caused a significant ($p < 0.001$) retardation of wound healing. In SC, but not TC this was accompanied by significant down regulation in TNF- α , IL6 and IL1b expression. However in stroma cells there was down regulation in the expression of TNF- α . These findings suggest a positive role for SLPI in ovarian tissue turnover, likely associated with increased expression of pro-inflammatory cytokine. Glister C et al (2014) BMC Genomics 15:72.

SRF.4 The metabolic and developmental impact of murine embryo culture in a novel microfluidic device

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Mammalian embryos are exquisitely sensitive to the in vitro culture environment, which must support cell division, metabolism, and genetic and epigenetic development. Microfluidic devices offer a mechanism to control this environment, potentially improving in vitro embryo development and quality. We report the optimisation and developmental impact of a recently developed polydimethylsiloxane microfluidic device for in vitro culture of murine embryos to the blastocyst stage^[1]. To test the impact of microfluidic culture on embryo developmental competence, cryopreserved C57BL/6N mouse zygotes (MRC Harwell, UK) were thawed and cultured in groups of 8-10 in 400nl chamber devices or control drops under oil (1 μ l media/embryo) at 37°C, 5%CO₂/5%O₂/90%N₂. After 72hr, embryos were removed to individual 4 μ l drops for 24hr to profile glucose, pyruvate and lactate turnover^[2]. Blastocysts were subsequently transferred to fibronectin-coated dishes for 72hr to evaluate attachment and outgrowth^[3]. To define the limitations of microfluidic culture, parallel groups of 10-40 2 cell mouse embryos were cultured to the blastocyst stage before metabolic profiling.

Microfluidic culture was non-embryotoxic and similar blastocyst formation, hatching, attachment and outgrowth rates were achieved between devices and controls ($n=15/15$, $p > 0.05$). However, individual blastocyst pyruvate consumption reduced following microfluidic culture (8.4 ± 0.6 , $n=139$) vs controls (10.9 ± 0.5 pmol/embryo/hr, $n=144$, $p < 0.0001$), while glucose consumption significantly increased in device blastocysts (7.2 ± 0.6 pmol/embryo/hr, $n=139$) vs controls (5.2 ± 0.5 pmol/embryo/hr, $n=144$, $p = 0.004$). Energy substrate turnover did not predict blastocyst outgrowth capacity in either system.

Blastocyst hatching rate in devices significantly decreased with increased group size (40/group: $2.2 \pm 2\%$ compared to 10/group: $30 \pm 4\%$, $n=4$, $p = 0.02$). Embryos cultured in groups of 40 had significantly reduced pyruvate (0.37 ± 0.1 pmol/embryo/hr) and glucose consumption (0.05 ± 0.03 pmol/embryo/hr, $n=3$) than groups of 10 (1.4 ± 0.08 pmol/embryo/hr, and 0.8 ± 0.08 pmol/embryo/hr, $n=3$, respectively $p = 0.02$).

Murine embryo developmental competence and metabolism were comparable between novel microfluidic device and conventional drop culture systems. Further validation of microfluidic culture efficacy will be provided through ongoing embryo transfer trials.

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Short papers session 2A: ACE post-registration prize (inc Australian SIRT exchange prize) - part sponsored by CooperSurgical

SP2A.1 A systematic literature review to determine whether "eggs that have failed to fertilise by normal IVF or ICSI are not re-inseminated by any means" is in the best interest of patients

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Background: HFEA code of practice states that "eggs that have failed to fertilise by normal IVF or ICSI are not re-inseminated by any means". This contradicts standard practice in countries outside the UK where rescue ICSI is performed routinely on day 0 on cycles where most oocytes fail to extrude a second polar body by ~6 hours post sperm-oocyte co-incubation, as a strategy to avoid failed fertilisation.

Aims: This study aimed to review the evidence base assessing the feasibility, efficacy and safety of early rescue ICSI.

Methods: PRISMA method was used to assess relevant publications identified via PUBMED and within the reference list of relevant publications from 1992 to August 2018. Day 1 rescue ICSI studies were not within the scope of this study and were excluded. Main outcome measure was clinical pregnancy rate, with secondary outcome measures: fertilisation, polyploidy and malformation rates. 14 studies, including 2475 cycles (with 1377 transfers) fit the inclusion criteria, with the following pooled 2PN fertilisation (68%,11022/16101), polyploidy (6%,949/15287), clinical pregnancy (45%,620/1377), implantation rate (30%,647/2124) and malformation per live birth rate (0.3%,2/711).

Conclusions: The literature reports that early rescue ICSI lead to the healthy live birth of at least 709 babies, without any reports of elevated rate of malformations. Although the live birth rate is potentially reduced compared to normal ICSI, it is a preferred alternative for patients instead of a failed cycle. It is estimated that if this practice were to be allowed in the UK, an additional 684 babies would be born per year to couples who, following the current HFEA guidelines, are instead left with the consequences of a failed cycle outcome. The pros and cons of adapting the HFEA code of practice to allow rescue ICSI are discussed, as well as the inherent ethical considerations of implementing the amendments now versus awaiting additional evidence.

SP2A.2 Managing expectations of patients with a single oocyte collected - a retrospective analysis of outcome

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Aims: In assisted reproduction cycles, the criteria to proceed to collection is commonly the observation of ≥ 3 follicles above $\geq 18\text{mm}$ diameter, but for some women with low ovarian reserve the best they may achieve is 1-2 follicles of this size. This review aims to provide outcome data for patients who obtained a single oocyte, to better manage patient expectations and provide appropriate support following retrieval.

Method: 487 cycles (2012-2017) with a single oocyte collected, were retrospectively analysed. Oocyte donation recipients and cycles with genetic testing were excluded. Patients were grouped based on age; group A < 37 , group B 37-40, group C > 40 . The outcomes measured were positive hCG, clinical pregnancy (CP) and live birth (LB) rates.

Results: Of the 487 oocytes collected, 91.2% were mature. 43.9% of cycles underwent standard *in vitro* fertilisation and 46.1% ICSI with 59% and 67% fertilisation rate respectively. 93.6% of these cleaved and 42.7% were transferred on day 2 post oocyte recovery, 56.9% on day 3 and 0.4% on day 5. These results were similar across the 3 groups. Predictably, the best outcomes, per embryo transfer were found in group A; positive hCG 41.1%, CP 32.1%, LB 26.3%. This was compared to 28.1% positive hCG, 22.9% CP and 19.8% LB in group B and 10.8% positive hCG, 6.3% CP and 3.6% LB in group C.

The outcomes for patients ≤ 40 years are respectable, with at least 1 in 5 patients achieving a live birth per embryo transfer, but as expected, live birth rate for patients over 40 with a single oocyte are low. It is vital that patients are given honest and realistic expectations when undertaking their treatment and should be supported to make decisions based on accurate clinical data. Other alternatives to IVF/ICSI, such as oocyte donation may be more appropriate and should be discussed at consultation.

SP2A.3 Pre-vitrification expansion stage does not appear to influence the FET outcome

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Objectives/background: Individual patient's blastocysts are frequently cryopreserved as a mixed cohort, with no traceability between single straws and indicative grades. This study aimed to establish the optimum blastocyst grade prior to vitrification, based on resultant post-warming survival, re-expansion status, quality and ability to implant.

Methods: A retrospective analysis of individually traceable, vitrified day-5 blastocysts (n=56), was conducted, graded as per NEQAS (numeric scoring; expansion, inner cell mass (ICM), trophectoderm (TE)) and subsequently vitrified, without artificial collapse. Warmed blastocysts' suitability for transfer was assessed (survival, re-expansion) within 1-hour. There were 42 single- and 7 double-transfers with a 44.6% implantation rate. General linear regression modelling was used to correlate pre-vitrification blastocyst grading to post-warming re-expansion, quality and implantation. ANOVA and t-test were used to detect differences between groups. $p < 0.05$ regarded as significant.

Results: Post-thaw quality (TE+ICM) was significantly correlated with pre-freeze quality (f4.8; $p = 0.02$), as expected, and most significantly predicted by pre-freeze TE ($p = 0.004$). Blastocysts more expanded pre-freeze were less likely to fully recover their expansion status post-thaw ($t = -3.9$; $p < 0.001$), but the higher the cohesiveness of ICM, the higher the likelihood of re-expansion ($p = 0.012$). However, lack of full re-expansion did not affect implantation potential, with pre-freeze grade (TE+ICM, $p = 0.02$) being the most important predictor variable for implantation.

Conclusions: Both ICM and TE have a differing effect on post-thaw quality; higher initial TE score should be favoured for freezing. Less expanded blastocysts prior to freezing, and those retaining a cohesive ICM post-warming, are both associated with increased likelihood of re-expansion; this should not however guide selection for transfer, as overall quality was shown to be more predictive of outcome. Conclusions are limited based on the sample size and may differ if blastocysts were artificially collapsed. FET outcome appears to be based on cell quality and not pre-vitrification expansion stage.

SP2A.4 An early chronotype in women is associated with IVF pregnancy

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Background: This study assesses the relationship between chronotype (morningness/eveningness) of female IVF patients using objective measures. Night shifts, jet lag and other diurnal disturbances adversely affect fertility. Female fertility relies upon clock genes, however, there has been little research into assisted conception outcomes in relation to patient chronotype.

Methods: Following HRA approval, 120 female patients (average age 35, range 23-45) completed the Munich Chronotype Questionnaire ahead of their IVF cycle, reporting times of waking/sleeping, age, height, weight, caffeine intake, smoking, exercise, daylight exposure. Patients' non-working day responses were used to determine mid-sleep time to indicate chronotype. IVF/ICSI cycles were monitored for pregnancy. Embryoscope time-lapse facilitated objective measurements of embryo development. We used time to 2-cell, 8-cell and blastocyst to indicate embryo developmental speed. Associations between morphokinetics, pregnancy and questionnaire responses were analysed.

Results: There was a significant association between early chronotype and pregnancy (urinary test and fetal heart, $p < 0.05$). Optimal mid-sleep time (73% pregnancy rate) was 02.30-03.30am. Follow-up to birth is ongoing. For the best 3 embryos per woman, increasing BMI positively correlated with average time to 2-cell ($p = 0.004$) and 8-cell ($p = 0.006$), but not blastocyst. Increasing female age positively correlated with time to 8-cell for the best 3 embryos ($p = 0.021$). The average preferred mid-sleep time in this study was 3.14am, slightly earlier than women of similar ages in the general population (4.11am $\pm$ 2.12 to 3.35am $\pm$ 2.28) (NS). As expected, morphokinetic criteria showed that ICSI embryos developed faster than IVF embryos, however, despite known associations between eveningness and higher BMI, we found no association between chronotype and times to 2-cell, 8-cell or blastocyst. **Conclusions** 1. Pregnancy after IVF is associated with early chronotype in women. 2. Cleavage to 2-cells and 8-cells slows as female BMI increases, but blastocyst formation is not delayed. Chronotype is not routinely addressed in lifestyle advice but deserves further investigation.

SP2A.5 Day of embryo biopsy and genetic diagnosis: Is there a link?

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Objective: To establish if there is a difference in the number of euploid embryos obtained following day five or day six trophectoderm biopsy.

Methods: Trophectoderm biopsy was performed on day five or six following assisted hatching on day four of development using a laser (RI Saturn). Embryos were deemed suitable for biopsy if the expansion had caused the blastocyst to herniate $> 40\%$ and the trophectoderm and inner cell mass was graded B or above as per the Gardner grading system. Chromosomal analysis of the cells was performed using NGS at an external laboratory (CooperGenomics). Genetic results were reviewed and outcomes were categorised by day of biopsy and age of the patient. The data was analysed retrospectively for statistical significance.

Results: 609 embryos were analysed in total for 192 patients over a period of 17 months. In total, 159 embryos were found to be euploid on day 5 (26%) and 99 on day 6 (16%) across all age groups. This result showed no statistical significance (p -value 0.65). The aneuploidy rate on day 5 was found to be 26% and 24% on day 6. Again, this result showed no statistical

significance (p-value 0.45). The mosaic rates were 3% (day 5) and 2% (day 6) whilst the no result diagnosis were 0.5% (day 5) and 1.1% (day 6). The euploid results on day 5 and 6 for patients <38 years were 59% and 46% respectively (p-value 0.31), for 38-40 year olds the rates were 38% and 55% (p-value 0.40), for 40-42 year olds the rates were 25% and 16% (p-value 0.36) and for >42 year olds they were 14% and 8% (p-value 0.43). No statistical significance was observed among any age group.

Conclusions: Whilst our data set is small, it has shown that there is no link between day of biopsy and the embryos genetic result. We can conclude that whilst some embryos may develop more slowly and are only suitable for biopsy on day 6, they are just as probable to have a euploid diagnosis and are just as valuable.

SP2A.6 The development and use of a laboratory prognostic tool for IVF patient success

Catherine Wass; Isaac Evbuomwan; Kirsten Keenan; Laura Irving

Gateshead Fertility Unit, UK

Background: Female age is an important predictor for IVF success^[1], and success rates are traditionally stratified according to female age. However, there are numerous other factors which can also affect the likelihood of achieving a pregnancy^[2,c]. Stratifying patients more comprehensively can aid analysis of laboratory performance and audit of policy changes. A "Traffic Light" System (TLS) was developed which can be used to predict IVF success based on multiple clinical factors.

Methods: The TLS was designed to predict IVF success based on factors that are uninfluenced by laboratory environment or processes e.g. embryo quality, so can be used as an audit tool to retrospectively analyse laboratory performance and the impact of any changes to protocol or equipment. The TLS includes; female age, FSH dose, number of eggs collected, aetiology, and fresh cycle number. Each category is subdivided into "green", "amber" and "red", weighted, and allocated 0,1 or 2 points respectively. The points are added to give each patient a final score; 0-3 = "green" patient and good prognosis for achieving an ongoing pregnancy, 4-6 = "amber" patient and average prognosis, 7+ = "red" patient and poor prognosis.

Impact: Examples of use:

- Identifying which patients should be targeted for SET
- Auditing outcomes after implementing blastocyst culture and time lapse imaging
- Troubleshooting dips in pregnancy rates and increased pregnancy loss
- Troubleshooting low success rates in certain age groups
- Auditing whether a strict SET policy is detrimental to certain patient groups.

Conclusion: The TLS has been in use since 2012 and has proved a useful tool when auditing multiple aspects of the Unit's performance and service development. Historically, it has been used retrospectively, but is now also being considered as a prospective tool, e.g in identifying poor prognosis patients who may benefit from having two embryos transferred.

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Short papers session 2C: Offspring

SP2C.2 The impact of paternal low protein diet and methyl-donor supplementation on fetal growth and placental development

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Background: There is increasing evidence that poor paternal nutrition pre-conception can increase the likelihood of his offspring developing cardiovascular and metabolic disorders in later life. Correct placental development and function are fundamental to positive offspring outcomes, however, there is emerging evidence that poor paternal diet can negatively affect placental development. This study aimed to determine how paternal low protein diet in mice influences the placenta and whether dietary interventions can correct any impairments.

Methods: Male 8-week old C57BL6 mice were fed a diet containing either normal protein (NPD; 18% casein), low protein (LPD; 9% casein) or low protein diet supplemented with methyl-donors (MD-LPD) for 8 weeks. Males were time mated with 8-9 week-old virgin female C57BL6 mice. Fetal and placental weights were measured at embryonic day 17 and placental tissue was collected for morphological and gene expression analysis.

Results: Paternal LPD increased fetal weight compared to NPD, whereas MD-LPD weights were similar to NPD. However, LPD and MD-LPD demonstrated reduced placental weight compared to NPD. Histological analysis revealed LPD and NPD placentas had similar gross-morphology, whereas MD-LPD placentas displayed increased thickness and junctional zone area compared to NPD and LPD placentas. Decidual and junctional zones of MD-LPD placentas had increased glycogen content

compared to NPD. Paternal diet had no significant impact on the expression of genes involved in glycogen synthesis and breakdown in the late gestation placenta.

Conclusion: This study provides evidence that sub-optimal paternal diet impacts on placental development and fetal growth. Furthermore, while supplementation of male LPD with methyl-donors prevented fetal overgrowth, placental weight and morphology were still altered. Further detailed investigations are needed to determine fully the underlying molecular mechanisms linking paternal diet with fetal growth and placental function. In addition, the impact of paternal dietary supplementation on fetal and placental development also requires further examination.

SP2C.3 Zinc supplementation during in vitro culture of bovine growing oocytes

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Background: In cattle, early antral ovarian follicles (0.5-2 mm in diameter) typically contain growing oocytes. Most of these oocytes have filamentous chromatin within the germinal vesicle, are still transcriptionally active and have not yet acquired the meiotic competence, as demonstrated by the inability of resuming meiosis once isolated from the follicular compartment. Consequently, these oocytes cannot currently be used in standard protocols for in vitro embryo production (IVP) limiting the exploitation of the ovarian population for assisted reproduction purposes.

Aims: In this view, aim of our studies is to improve the outcome of in vitro culture of growing oocytes (IVCO), in order to increase the amount of oocytes per ovary that can be used for embryo production.

Method: By mining transcriptomic databases (<http://emb-bioinfo.fsaa.ulaval.ca/IMAGE/>) we observed that zinc (Zn) transporters are differentially expressed in different cell types throughout several steps of oogenesis in cattle. This observation, supported by studies in mice that revealed the importance of Zn in regulating oogenesis and early embryogenesis, seems to suggest the supplementation of culture medium with this element as a possible strategy. Hence we tested whether Zn might be beneficial to the acquisition of meiotic competence in bovine growing oocytes.

Results: Zn supplementation during 24 hours of IVCO significantly improved the proportion of oocytes reaching the metaphase-II stage of meiosis after subsequent standard in vitro maturation compared to non-supplemented control. Furthermore the global transcriptional activity was significantly increased in growing oocytes cultured with Zn, while short time treatment with a Zn chelator (TPEN) had a negative effect on transcription. From a mechanistic point of view, these findings likely suggest that Zn support the synthesis of molecules needed for the successful completion of the subsequent steps of oogenesis in growing oocytes. Further studies are in progress to test the effect of Zn supplementation on embryo development of growing oocytes.

SP2C.4 On-chip mouse embryo culture: Evaluation of effects of uterine cells-conditioned media on embryo development and gene expression

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Background: Microfluidics has recently been proposed as a method to overcome the limitations of traditional oocyte and embryo culture methods. In this work, we report the use of a microfluidic polydimethylsiloxane device as promising alternative for in vitro embryo culture, and we have evaluated the effects of cells-conditioned media (CM) on embryo development.

Methods: The microfluidic device was fabricated using traditional soft-lithographic technique. To produce CM, mouse uterine epithelial cells (Creative Bioarray, USA) were cultured in KSOM (Merck Millipore, UK) for 24 h. The CM was used to culture groups of 12, 1 cell murine embryos (B6C3F1xB6D2F1 strain, EmbryoTech, USA) into a microfluidic device. Control embryos were cultured in the device using KSOM. We compared blastocyst rates of embryos cultured in CM with those obtained using KSOM. The effect of treatment on embryo gene expression was assessed in cDNAs generated from individual stage matched, blastocysts using a custom, real time PCR array.

Results: Developmental ability of mouse embryos in the presence of CM was significantly higher ($p < 0.05$) in comparison with control media. Blastocyst rates for the CM ($n = 15$ devices, 180 embryos) and control media ($n = 15$ devices, 180 embryos) groups were 68.9% and 45.1%, respectively. qPCR results showed that expression of Makorin Ring Finger Protein (MKRN, $p = 0.036$), DNA Methyltransferase 3 β (DNMT3 β , $p = 0.012$), DNA (Cytosine-5-)-Methyltransferase 3-Like (DNMT3L, $p = 0.043$), Histone Acetyltransferase 1 (HAT1, $p = 0.006$), Keratin 18 (KRT18, $p = 0.028$), and Ubiquitin Like With PHD And Ring Finger Domains 1 (UHRF1, $p = 0.043$) was significantly different between the treatment groups. Specifically, we observed in the CM group increased expression of DNMT3 β and DNMT3L, which play an important role in early embryo development.

Conclusions: These findings revealed that the new microfluidic device supports mouse preimplantation embryo development in vitro. Uterine epithelial cells-conditioned medium has the potential to enhance blastocyst development. Further investigations are required to identify the mechanism of this effect.

SP2C.5 Placental endocrine malfunction induces reproductive defects in murine offspring postnatally

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Background: The placenta is vital for pregnancy. It transports nutrients to the fetus and secretes hormones that adapt maternal physiology to support fetal nutrient supply. Impaired placental function disrupts materno-fetal nutrient allocation and results in pregnancy complications, with long-lasting impacts on offspring cardio-metabolic function. However, little is known about the importance of placental endocrine function on offspring reproductive function.

Method/aims: We have established a mouse model where placental endocrine function is selectively modified by conditionally deleting the expression of insulin-like growth factor-2 (Jz-Igf2-loss). Recent work has found that this leads to fetal growth restriction and metabolic dysfunction of female offspring postnatally. We hypothesised that Jz-Igf2-loss females will also have reproductive defects. Vaginal opening was recorded and smears were then obtained from 8 week old control and Jz-Igf2-loss females over 14 days to assess their estrous cyclicity. At 13 weeks, offspring were sacrificed and ovaries weighed and processed for histology. Sections of ovaries from mice in proestrus were stained with haematoxylin and eosin and the number of primordial, primary, secondary, tertiary and atretic follicles, and corpus luteum quantified.

Results: Vaginal opening occurred earlier in Jz-Igf2-loss females versus controls (29.4 ± 0.7 vs 35.7 ± 0.7 days < 0.001). Jz-Igf2-loss females also displayed longer estrous cycles (Jz-Igf2-loss 5.7 ± 0.2 versus control 5.1 ± 0.1 days, $p < 0.05$) due to an 80% greater time spent in estrus ($p < 0.05$). Ovaries from Jz-Igf2-loss females were 23% heavier and contained more atretic and fused follicles but fewer secondary and tertiary follicles compared to controls ($p < 0.05$). This is the first study to show placental endocrine malfunction programs changes in the reproductive system of female offspring.

Conclusions: The earlier vaginal opening and increased time in estrus may reflect an attempt to increase reproductive fitness, which may however, have come at the expense of normal folliculogenesis in Jz-Igf2 loss females.

Short papers session 2D: Andrology (including Iwan Lewis-Jones Prize)

SP2D.1 Protective effects of omega-3 fatty acids on cyclophosphamide induced fertility alterations

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This study evaluated the protective effect of omega-3 fatty acids (O3FA) on cyclophosphamide (CPP)-induced fertility alterations. It was motivated by the need to reverse the induced infertility (especially gonadal toxicity/dysfunction), other forms of reprotoxicity and teratogenicity effect caused by chemotherapy drugs. Eighty-four sexually mature male and female rats were used to demonstrate CPP-induced fertility alterations with regards to sperm counts, histology of the gonads, conception rate, early developmental parameters (live fetal numbers, litter size, foetal weight, copora lutea number, fetal crown rump length, resorbed embryo number and implantation), fertility index, resorption index, implantation index and O3FA possible protective effects. All histological examinations were conducted by preparing slides and capturing the micrographs while sperm cells were counted using haemocytometer. Omega-3 fatty acids ameliorated the adverse effects of CPP in male rats by causing an increase in relative testicular weights, reduction of missing germ cell layers, reduction in atrophy of seminiferous tubules, increase in testicular and epididymis sperm counts. Ovary micrographs indicated that O3FA ameliorated the degeneration of pre-ovulatory follicles and consequent formation of atretic follicles caused by CPP. The CPP + O3FA-treated female rats showed significant increase ($p < 0.05$) in conception rate, mean fetal weight, copora lutea number, fetal crown rump length and fertility index compared with rats treated CPP only. In conclusion, O3FA exhibited protective effects in cyclophosphamide-induced fertility alterations. Keywords: omega-3 fatty acids, cyclophosphamide, fertility, infertility

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SP2D.2 Trace element profiles in canine and human semen: Evidence that dietary intake of micronutrients may influence semen composition and sperm function?

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Background: The influence of diet, particularly trace element status, on male fertility is controversial. Trials of mineral supplementation have been inconclusive. Here, we have taken a multi-species approach to characterise seminal fluid elemental composition in a) groups of fertile or sub-fertile men and b) groups of dogs routinely fed widely-different diets.

Materials and methods: Human study subjects were categorised as being fertile, sub-fertile or sterile (vasectomised). Each voluntarily donated semen to a fertility clinic. Canine samples were obtained as part of routine fertility-checking procedures from dogs fed either a raw, unrefined carcass-based diet (RAW) or a commercially-available, refined and nutritionally-balanced diet for dogs (REFINED). Semen was analysed for sperm characteristics (motility, concentration) and seminal fluid for 32 trace elements by inductively-coupled plasma (ICP) mass-spectrometry.

Results: Overall elemental composition of human semen was similar in all groups of healthy men e.g. no difference in Zn, P, K, Na, Mg, Se which together constitute 99% of seminal fluid elements. Further analyses indicated low chromium ($p<0.001$) and manganese ($p<0.001$) but high molybdenum ($p<0.002$) in subfertile, relative to fertile, patients. In canines, where separate prostatic vs testicular fractions may be collected, extremes of diet significantly altered seminal elemental composition in both fractions (lower for all elements, with the exception of iron, in RAW vs REFINED) and confirmed higher Zn in the prostatic-rich fraction ($p<0.01$).

Conclusions: Zn and Se supplementation in humans is unlikely to have direct effects on sperm development and structure since these trace elements are either from the prostate (Zn) or are not influenced by diet (Se). Variation in seminal Cr, Mn and Mo may have a role in sperm function. Extremes of diet (albeit in canines) can influence seminal fluid composition. Further proof-of-principle clinical studies are warranted.

SP2D.3 Structural modification of pentoxifylline improves sperm motility and fertilization rate and developmental competence of embryos

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Background: Poor sperm motility is common cause for fertilization failure in assisted reproductive technology (ART). Motility enhancement using nonspecific inhibitors of phosphodiesterase enzyme is a common approach. Pentoxifylline (PTF) is most commonly used as a potent sperm motility enhancer in ART. However, there are reports in the literature suggesting its adverse effect on fertilization and embryonic development. The present study was intended to see whether modifying the structure of pentoxifylline can enhance the sperm motility without having any adverse effect on the fertilization potential of spermatozoa and subsequent embryo developmental potential.

Objective: To assess the effect of structurally modified pentoxifylline on sperm motility, fertilization rate and developmental potential of early embryos.

Methods: We synthesized 8 structurally modified pentoxifyllines (mPTF 1 to mPTF 8) compounds and purity of these compounds was confirmed by infra red (IR) and mass spectral (MS) analysis. These compounds were assessed for beneficial effect on human sperm motility among which mPTF1 (One-[(6E)-7-(6-methoxynaphthyl)-5-oxohept-6-en-1-yl]-3,7-dimethyl-3,7-dihydro-1H-purine-2,6-dione) exhibited superior motility enhancement. The functional competence of spermatozoa was assessed by CASA, mitochondrial function, DNA integrity and acrosome reaction in human spermatozoa. To determine whether mPTF1 has any adverse effect on fertilization and embryo development we performed in vitro fertilization (IVF) using mouse model. The caudal sperm from male mice were incubated in EBSS medium with bovine serum albumin (BSA, 2.5%) containing PTF (1mM) or mPTF1 (0.25mM) and IVF was performed from these spermatozoa. The fertilization and cleavage rate was assessed. The DNA integrity of the embryo quality was assessed by using TUNEL assay.

Results: When spermatozoa were incubated in Earls balanced salt solution (EBSS) with or without PTF (1 mM) or mPTF1 (0.25 mM), the progressive motility of the spermatozoa was higher in mPTF1 group at all intervals. Processing spermatozoa with mPTF1 did not induce any mitochondrial membrane damage and DNA damage to the spermatozoa with respect to control. IVF results indicated that fertilization rate (82.8%) and embryo developmental competence (Blastocyst rate:74%) are better in than in PTF group (Fertilization rate: 71.8%, blastocyst rate: 65.5% respectively). The DNA damage of the embryos were lower in mPTF1 (6.3%) group compared to PTF (9.8%) group further indicating that the quality of the embryos derived from mPTF1 exposed spermatozoa is not compromised.

Conclusion: Structural modification of pentoxifylline can improve the sperm functional competence, fertilization rate without affecting the quality of embryo.

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SP2D.4 Defining the impact of paternal diet on testicular morphology and apoptosis

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The adverse effects of poor diet on sperm quality are well recognized. It is conceivable that underlying any impact on sperm quality will be a change in testicular morphology. However, few studies assess the impact of a male's diet on the histology of his testes. Therefore, we fed male C57BL6 mice either a control normal protein diet (18% protein; NPD), isocaloric low protein diet (9% protein; LPD) or a LPD supplemented with methyl-donors (MD-LPD) for at least 7 weeks. Testes were

collected and processed either for analysis of morphology (histology) or gene expression (RT-qPCR). We observed that testes from LPD fed males displayed increased mean seminiferous tubule area, perimeter and area of seminiferous epithelium when compared to NPD and MD-LPD ($P < 0.02$). In contrast, testes from MD-LPD fed males displayed an increased area of the seminiferous tubule lumen when compared to NPD male testes ($P = 0.02$). To determine the underlying molecular mechanisms linking diet with testicular morphology, we analysed the expression of several pro- and anti-apoptotic regulating genes by RT-qPCR. We observed that in MD-LPD testes, the expression of the pro-apoptotic regulator Bax (Apoptosis regulator BAX) was significantly decreased relative to NPD and LPD testes ($P = 0.01$). Furthermore, the expression of the anti-apoptotic regulator Bcl2 (Apoptosis regulator Bcl-2) was significantly increased in MD-LPD and LPD testes relative to NPD testes ($P < 0.05$). Finally, we analysed levels of testicular apoptosis using TUNNEL staining. Here, we observed significantly reduced levels of testicular apoptosis in NPD testes when compared to LPD testes ($P = 0.014$). These data demonstrated poor paternal diet impacts significantly on testicular morphology and levels of apoptosis. Furthermore, supplementation of the LPD with specific vitamins and methyl donors mitigated most, but not all, of these changes. These data highlight the importance of diet in regulating testicular morphology and function.

SP2D.5 The case for semen sampling as a simple, non-invasive surrogate for prostate health screening

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Background: Where present, the detection rate for prostate cancer (pCa) by invasive biopsy is high, fully justifying its use in confirmatory testing. However, because prior screening tests are relatively insensitive, false-positives are not infrequent, leading to unnecessary surgery with its associated complications. Despite the generally straightforward acquisition of samples, the ejaculate is rarely used to assess the health of the prostate. We hypothesised that the presence of prostatic cells and/or qualitative/quantitative differences in the RNA content of the ejaculate could change this paradigm.

Methods: Semen samples obtained from fertile and vasectomised men and from men with pCa were subjected to density gradient centrifugation (DGC) on a 60% cushion of Suprasperm™ and fractions containing somatic cells were washed and cytopun on to coated slides prior to fixation and immunocytochemistry with antibodies recognising prostatic markers. RNA was also isolated from these samples and prostate-specific markers were analysed by qRT-PCR. RNA sequencing libraries from seminal plasma were prepared corresponding to the 18-23 nt and 30-35 nt RNA size ranges. Libraries were interrogated by next generation sequencing and analysed to generate lists of differentially expressed RNAs associated with cancer and sterility.

Results: Cells expressing Prostate Specific Membrane Antigen and CD24 were observed in most DGC-processed ejaculate samples by indirect immunocytochemistry and by targeted qRT-PCR, supporting their enrichment and designation as prostatic epithelial cells. Small RNAs were highly heterogeneous in ejaculate semen, although tRNAs and 5SRNAs were the dominant forms. A few differentially expressed RNAs from these samples included hsa-miRNA-143, targeting many genes found in the KEGG pathway for pCa.

Conclusions: Although the small sample size for pCa limited the scope of the study, the outcomes strongly support a wider appraisal of ejaculate semen as a non-invasive surrogate of the prostate for monitoring prostate health, particularly among younger men.

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Short papers session 2E: Maternal factors

SP2E.1 Acute maternal high fat diet during preimplantation alone affects adult offspring hepatic expression of metabolic and epigenetic markers

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We examined the impact of maternal short-term high fat diet (HF) before implantation on expression levels of metabolic and epigenetic markers in progeny livers in a murine model. Mice were mated at 7-8.5 weeks and randomly assigned to 3 groups: a) HF diet (45% kcal fat, 20% kcal protein, 35% kcal carbohydrate) from day of plug to d3.5 (embHF); b) HF diet throughout gestation and lactation (HF) and c) standard diet (21% kcal fat, 17% kcal protein, 63% kcal carbohydrate; control).

Total RNA was extracted from livers (n=49) of 28 week old offspring (RNeasy Lipid Tissue Mini Kit, QIAGEN, UK), cDNA synthesized with random priming (ImProm-II™ Reverse Transcription System, Promega, UK) followed by qPCR triplicate reactions (SybrGreen Precision qPCR Master Mix, Primer Design) with Pck1 and Tbp as reference genes (geNorm).

Relative expression (dCt) was compared using a multilevel random effects regression model accounting for different parameters from individual animals (SPSS). Female offspring from HF mothers had smaller livers relative to bodyweight than control offspring ($p < 0.05$; 0.03 vs 0.04). Male offspring hepatic gene expression was less affected by maternal diet with an increase in Pck2 in HF.

In females, a graded response to maternal diet was observed with maternal HF displaying the most widespread impact. Markers for carbohydrate and lipid metabolism (e.g. Pck2, Sdha, Ppib, Faah, Ppara α , and δ , Lipin3, AdipoR1 and 2, InsR, Igf1, Igfr1) as well as epigenetic markers (Dnmt3a and b, Hdac1 and 3) were upregulated alongside structural genes (Tuba1) in HF female offspring ($p < 0.05$). Four of these genes (Pck2, Faah, Ppara α and Hdac3) were also significantly upregulated in the embHF offspring ($p < 0.05$).

In conclusion, acute maternal (just 3.5 days) HF diet without obesity experienced during the preimplantation period can compromise liver metabolism of glucose, lipids and some epigenetic marks, with female offspring being more sensitive than males.

SP2E.2 The risk of 'de novo' poor ovarian response during repeat IVF: Results of an externally validated prediction model based on more than 4,000 women

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Background: Although most research has focused on predicting poor ovarian response (POR) before the 1st IVF treatment, little is known about the risk of becoming a 'poor' responder during the IVF journey. The aim of this study was to identify factors relevant to the 1st IVF treatment that can predict POR during subsequent treatments.

Materials and methods: Consecutive patients who have undergone at least 2 oocyte retrievals during a 15-year period were retrospectively analysed. Women with up to 3 oocytes during 1st retrieval were excluded. Multivariable analysis in the form of logistic regression was performed to investigate the risk of experiencing POR (up to 3 oocytes) during the 2nd retrieval. Potential predictors were the number of oocytes (1st treatment), the stimulation dose (1st treatment), the woman's age (1st treatment) and the time interval between retrievals.

Results: Data from 4021 patients were analysed. All 4 parameters are independent predictors of subsequent POR; the number of oocytes obtained during the 1st retrieval is the most influential (unadjusted OR 0.77 95%CI 0.73 to 0.80, adjusted OR 0.78 95%CI 0.75 to 0.82). However, the prediction model combining all 4 parameters showed only marginal, non-significant improvement in discrimination (AUROC 0.80 95%CI 0.77 to 0.83) compared to the model that includes the number of oocytes only (AUROC 0.76 95%CI 0.73 to 0.79). The simpler model also maintained satisfactory discrimination when validated on an external population (AUROC 0.72) and, therefore, is ready-made for clinical use. According to this model, the optimal cut-off for identifying women at risk of subsequent POR is 8 or fewer retrieved oocytes.

Conclusion: Women with 4 - 8 oocytes during the 1st IVF treatment should be made aware of the risk of low ovarian response with subsequent treatments. This may further aggravate the negative impact of advancing age on the success of assisted reproduction technologies.

SP2E.3 Duration of ovarian stimulation does not affect the chances of live birth in couples undergoing IVF: an analysis of 8624 treatment cycles

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Purpose: To explore the association between the duration of ovarian stimulation and live birth rates (LBR) following IVF and identify the number of stimulation days that would optimise IVF outcome.

Methods: Anonymised data on all fresh IVF cycles (n=8624) performed in a tertiary fertility centre in the UK from January 2008 to December 2017 were obtained. Ovarian stimulation was undertaken using a standard agonist protocol. Primary outcome was LBR. Secondary outcomes were clinical pregnancy and miscarriage rates. Cycles were categorised into 5 groups according to the duration of ovarian stimulation (<8, 9-11, 12-14, 15-17 and >18 days). Outcomes were evaluated by group and further stratified by age group (<35, 35-37, 38-39, >40 years) and time period to account for improved LBR over time (2008-2010, 2011-2012, 2013-2014 and 2015-2017). Data were analyzed using logistic regression and controlled for age, duration of infertility, number of previous cycles and number of embryos transferred.

Results: A total of 8624 cycles were included. The median duration of ovarian stimulation was 12.5 days (SD=2.1). Univariable analysis showed no association between the duration of ovarian stimulation and LBR. Using multivariable analysis, clinical

pregnancy rates were significantly lower in cycles with >15 days of stimulation (OR 0.78, 95%CI 0.65-0.94, P 0.009). However, there was no significant association between days of ovarian stimulation and live birth (OR 0.99, 95%CI 0.96-1.01, P 0.247) or miscarriage rates (OR 0.98, 95%CI 0.94-1.02, P 0.415). The absence of the association between the duration of ovarian stimulation and LBR was consistent across all age groups and different time periods.

Conclusion: Whilst our analysis suggests that clinical pregnancy rate is higher in cycles with a shorter duration of stimulation (9-14 days), there was no association with live birth in women undergoing IVF treatment. Duration of ovarian stimulation in IVF is not a surrogate outcome for clinical success.

SP2E.4 Discriminative capacity of 1H-MRS spectrum binning for preterm delivery-associated Lactobacilli-dominated vaginal microbiota

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Background: Identification of vaginal microbiota community state types (CSTs) and concentrations of metabolites may offer better identification of asymptomatic women at risk of preterm delivery (PTD). We sought to determine whether spectrum binning of proton magnetic resonance spectroscopy (¹H-MRS) of cervicovaginal fluid (CVF) metabolite spectra improves the determination of specific vaginal microbiota CSTs and the prediction of PTD.

Methods: ¹H-MRS spectra from CVF of 300 asymptomatic women (20⁺⁰-22⁺⁶ weeks' gestation, who went on to deliver at term, n=250, and preterm, n=50) were binned at 0.02ppm. The dominant vaginal Lactobacillus microbiota CSTs (I, II, III, V) in a subset of 83 women were identified by sequencing the V1-V3 region of the 16S rRNA gene. Comparisons were made using two-way ANOVA, Receiver Operator Characteristics and PLS-DA.

Findings: Women with CST V (L. jensenii-dominated) had a significantly higher PTD rate (45.5%) compared to the total cohort (20%, p=0.004), and women with CST I (L. crispatus-dominated, 7.6%, p=0.02). Samples from CST I demonstrated higher spectrum bins containing lactate (1.28, 1.30 and 4.11ppm, p<0.0001) and glutamate peaks (2.38ppm, p=0.007) than other CST groups. Lactate (1.32 ppm), was predictive of PTD in both the CST I (AUC=0.98) and CST V (AUC=0.81) groups. Glutamate (2.40ppm) was predictive of PTD for CST I women (AUC=0.84). Women with CST I were discriminated from those with CST V by the spectrum bins containing the lactate (1.28ppm, AUC=0.84; 1.30ppm, AUC=0.83; 4.11ppm, AUC=0.85), glutamate (2.83ppm, AUC=0.81) and a combination of lactate and glutamate spectrum bins (AUC=0.94).

Conclusions: ¹H-MRS spectrum binning identified more predictive markers of PTD and shows promise for determining specific vaginal microbiota CSTs from CVF metabolite spectra. L. crispatus appears to be more frequently associated with stable vaginal microbiota and healthy pregnancy outcome, whereas L. jensenii appears to be associated with PTD in majority of cases.

SP2E.5 The effects of maternal hyperandrogenaemia on infant growth in infants born to mothers with PCOS

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Introduction: Epidemiological studies have found that infants born to women with PCOS are more likely to be either too small or too large for gestational age. Research suggests that infants born too small for gestational age, which display catch up growth in the first two years of life are predisposed to a plethora of adverse consequences such as obesity, type two diabetes and cardiovascular disease in later life.

Aims: The aims of the study were; to compare birthweight distributions, postnatal growth and the role of the hormones testosterone and IGF-1 in any observed differences in postnatal growth between babies born from mothers with PCOS and controls.

Methods: A prospective analysis of 79 infants at birth, 9 and 15 months of age and their mothers was undertaken to assess the growth and metabolic properties of infants born to mothers with PCOS.

Results: There were no significant differences in the outcomes of infants born to mothers with PCOS compared to controls. The mean birthweight (g) of infants born to mothers with PCOS are smaller (3360g) than those born to their control counterparts (3441g). With regards to the distribution of birthweight centiles, more infants were born SGA in the PCOS cohort compared to the controls (+6.5%). Mean testosterone levels were higher (+0.23 nmol/L) in PCOS infants born with a birthweight centile >=10th compared to controls. IGF-1 concentrations were lower in SGA infants born to the PCOS cohort compared to controls (-0.03pg/ml). Males display more catch up growth than females.

Discussion: The results obtained in this study alongside numerous others strongly indicate that the maternal intrauterine environment of a woman with PCOS is a risk factor for a developmental predisposition towards numerous long-term adverse outcomes in both the sons and daughters of these women.

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SP2E.6 Characterisation of vaginal cytokine expression during IVF: A cohort study

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Introduction: Reproductive functions such as endometrial receptivity and trophoblast invasion are closely regulated by the immune system. Hormonal changes following controlled ovarian hyperstimulation during IVF can influence the local immune response and therefore expression of vaginal cytokines and chemokines. Cytokine expression could thus be utilised as a non-invasive biomarker to improve cycle management and outcome.

Aims:

- To investigate changes in cytokine concentrations in the cervico-vaginal fluid (CVF) during IVF, in response to changes in plasma expression of oestradiol and progesterone.
- To identify differences in expression of local cytokines in fresh and frozen embryo transfer cycles; as well as successful and unsuccessful treatment outcomes.

Methods: Vaginal swabs and blood samples for E2 and P4 were collected from 53 patients at 6 stages during IVF. Vaginal samples were analysed using the Human Magnetic Luminex Screening assay for the presence of 17 pro- and anti-inflammatory cytokines known to be involved in the peri-implantation period and early pregnancy.

Results: A heatmap analysis indicated that groups of closely related cytokines had similar patterns of expression. Significant differences in expressions of IGFBP-1, IL-1B and IL-8 were observed throughout different stages of treatment. These changes corresponded with differences in serum levels of E2 and progesterone; IL-8 expression reduced as E2 levels increased from the start of pituitary suppression to the embryo transfer stage, indicating a reduction in inflammation at the time of implantation. IGFBP-1 was significantly higher at the time of embryo transfer for patients who reached clinical pregnancy. The overall difference in expression of analytes were not statistically significant between fresh and frozen embryo transfer cycles.

Conclusion: Local inflammatory responses are altered through different stages of IVF, correlating with plasma levels of E2 and progesterone. Fresh and frozen cycles produced similar cytokine profiles, suggesting the different protocols can elicit similar effects.

Short papers session 3A: Fertility preservation

SP3A.1 The effect of the chemotherapy agent Paclitaxel on the germline stem cell niche of the human immature testis

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Background: Whilst eradicating malignancies, paediatric oncological treatments can result in subfertility in men (1) indicating damage to either the germ cells and/or their somatic niche. Here, the effects of Paclitaxel (anti-tubulin used for refractory paediatric tumours) on the immature testis is investigated. The 2nd trimester human fetal testis consists of immature germ cells including gonocytes and (pre)spermatogonia, in addition to immature somatic cells and thus represents a suitable model for the human pre-pubertal testis.

Materials and methods: Human fetal testes (n=4, 14-22 gestational weeks) were dissected and placed in hanging drop culture at 37°C. Tissue was exposed to 25µM Paclitaxel or 0.1% DMSO (control) for 24 hours followed by chemotherapy-free media. Tissue was fixed in Bouins 24 or 96 hours post exposure. Double immunohistochemistry for gonocytes (anti-AP2γ) and (pre)spermatogonia (anti-MAGEA4), and Sertoli (anti-SOX9) and apoptotic (anti-cleaved caspase 3) cells was performed. Number of cells per testicular cord area (mm²) were counted and statistically analysed using two-way ANOVA. Immunohistochemistry was also performed for Anti-Mullerian Hormone (AMH) and Leydig cell steroidogenic enzyme CYP11A1.

Results: A significant reduction in gonocytes (504 v 295 cells/mm²; p=0.026) was observed 24 hours after paclitaxel exposure, whilst number of (pre)spermatogonia was unchanged (109 vs 176 cells/mm²; p=0.0809). At 96 hours post-exposure, a significant reduction in gonocytes (296 v 126 cells/mm²; p=0.004), (pre)spermatogonia (133 v 59 cells/mm²; p=0.0001) and Sertoli cells (4649 v 3556 cells/mm²; p=0.0036) was observed. No difference in apoptotic cells, AMH and CYP11A1 expression was noted.

Conclusion: Paclitaxel targets gonocytes and (pre)spermatogonia at different time points in the immature testis. The reduction in (pre)spermatogonia coincided with reduced Sertoli cell number, suggesting that germ cell loss is secondary to effects on its niche. The long-term effects of Paclitaxel and ability of the immature testis to recover is currently being investigated in human testicular xenografts.

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SP3A.2 Fertility preservation provision for female cancer patients in England

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Purpose/background/objectives: Fertility preservation (FP) has an increasingly important role in modern cancer care. However, there is little data available regarding actual provision of it in the UK. The aim of this study is to establish the national provision to assess compliance with national guidance and uptake of FP.

Methods: A Freedom of Information request was sent to all 209 Clinical Commissioning Groups (CCG) in England for information about the total number of funded FP cycles between 1st January 2015 and 31st December 2017, funding criteria, exceptional funding requirement, time to process the request and funded storage period.

Results: Response rate was 90%. However, only 26% of responders could provide information on the estimated number of funded FP cycles. The rest claimed not to have this information. The total estimated number of funded cycles reported in the studied period was 752 cycles. Furthermore, 89 CCGs have an upper age limits varying from 35 and 42. Sixty CCGs do not offer FP for women who have a living child. 54 CCGs require exceptional funding request form with significant variation in the time to process ranging from 1 to 56 days. The duration of funded storage also varied between 1 to 10 years. 4 CCGs do not offer oocyte preservation and 22 CCGs do not offer FP if patients had previous NHS-funded IVF cycles.

Conclusions: This study demonstrates that FP provision in women with cancer is very inconsistent throughout most of the country. The vast majority of CCGs currently continued to apply general IVF criteria for FP patients, despite revised 2013 NICE guideline. With 75,000 young women diagnosed with cancer during the same period, this study confirms the gross under-representation of patients who benefited from this treatment due to restrictions and funding.

Short papers session 3B: The embryo

SP3B.1 The quality of the overall cohort of embryos at day 3 is more predictive of success than quantity

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Background: Embryo quality is an important factor in assisted conception success (1-2), however, quality may be compromised in favour of quantity. We hypothesise that the overall quality of the cohort of embryos, rather than the quantity retrieved is more predictive of successful outcomes.

Methods: A retrospective cohort study of 1484 consecutive women undergoing IVF/ICSI at a single centre.

Results: Fresh IVF/ICSI cycle outcome and pregnancy data was collected in 1217 patients. 318 patients had a high proportion (>50%) of high-grade day 3 embryos with a total embryo yield < 5 (group A), and 90 had a high proportion of high-grade day 3 embryos, with a total embryo yield of > 15 (group B). There was no statistically significant difference in pregnancy rate (χ^2 2.14, $p=0.2$), clinical pregnancy rate (χ^2 0.223, $p=0.7$) or live birth rate (25% vs 29%, χ^2 0.11, $p=0.8$) between the two groups. 358 patients had a low yield of embryos (<5). Of these, 40 cases had a low proportion of high-grade day 3 embryos (<50%, group C), and 318 had a high proportion of high-grade day 3 embryos (>50%, group D). Group D had a statistically significant higher live birth rate (25% versus 2.5%, $p=0.01$).

Conclusions: Our data shows that when comparing patients with a high and low yield of embryos there is no significant difference in live birth outcomes when the overall quality of the cohort of embryos at day 3 is high. Furthermore, in patients with a low yield of embryos, a difference of 1 or 2 high-grade embryos has a significant impact on the live birth rates. Our study lends weight to the argument that quality is the most important factor in determining outcomes in a fresh cycle and can help in counselling women with low antral follicle counts and AMH.

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SP3B.2 Day-6 vitrified blastocysts have similar birth outcomes to day-5 blastocysts but there is a tendency to higher birthweight and birth centile for gestational age in the day-6 cohort

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Background: Births after transfer of blastocyst embryos are associated with increased incidence of babies who are "large for gestational age" (LGA), possibly due to the prolonged embryo culture to day-5^[1,2]. Day-6 blastocysts, with their 20% longer culture, are frequently cryopreserved and transferred in a subsequent cycle. The aim of this study was to compare the perinatal outcomes after transfer of day-5 and day-6 vitrified blastocysts to examine the effect of that protracted *in vitro* culture.

Methods: Blastocysts were vitrified between September 2010 and December 2016 in a single centre. All were subsequently transferred as if they were day-5 blastocysts. Cycles where day-5 and day-6 blastocysts were transferred together and cycles involving donated oocytes were excluded.

Definitions: Small for gestational age (SGA) is birthweight <10th centile for expected gestation; LGA is birthweight > 90th centile (UK birthweight charts).

Results: The day-6 group comprised 177 cycles in 138 women. The day-5 group comprised 754 cycles in 580 women. There was no difference in BMI or circulating AMH but the day-6 group were older at the time of vitrification and subsequent FET (P<0.001). There was no difference in the live birth rate (39.1%vs.39.6%, P=0.908), mean birthweight (3558±594g vs. 3488±578g, P=0.417), birth centile (65±24 vs. 60±28, P=0.254), proportion of SGA (1.9%vs.5.1%, P=0.306) or LGA (18.5%vs.16.9%, P=0.778).

Conclusions: The singleton perinatal outcomes of the day-6 blastocysts were similar to day-5 blastocysts. However, both groups showed a higher mean birthweight centile than expected, in keeping with the published literature. There was a tendency for the birthweight in the day-6 blastocysts, with their increased culture media exposure, to be shifted even further to the right (heavier and proportionately fewer SGA and more LGA singletons). These data provide further support for the need for a larger dataset which may reveal a phenomenon that could have longer-term health implications.

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SP3B.3 In vivo fertilization: A more natural approach to IVF

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Background: Concern is growing that in-vitro culture may impact on epigenetic regulation of birthweight, growth and long-term cardiovascular health in babies born after IVF. The challenge is therefore to minimize the exposure of human gametes and embryos to synthetic conditions in this critical pre-implantation phase of development. A novel porous device (AneVivo™, Anecova SA, Switzerland) allows gametes and embryos to be placed into the uterine cavity, thus offering a natural and dynamic in-vivo environment for fertilization and early embryo development.

Method: Between June 2015 and August 2018 69 cycles using the device were performed in two centres. Half of the oocytes obtained (selected at random) were placed in the device, and half into the standard in-vitro environment for fertilization. ICSI was used in all cases. After 18 hours, in vivo fertilized zygotes were placed in in-vitro culture until selection for transfer. The best quality embryo was then selected regardless of whether it had undergone in-vivo or in-vitro fertilization. Outcomes analysed included fertilization, loss and degeneration of oocytes, embryo quality and pregnancy.

Results: There was no significant difference in in-vivo versus in-vitro fertilisation rates (66% vs 71%), oocyte degeneration rates (11% vs 13%) or percentage of top quality embryos (29% vs 35%). Clinical pregnancy rates were higher after the transfer of an in-vivo fertilized embryo: 81% vs 56%, but statistical significance was not demonstrated due to the cohort size.

Discussion: These preliminary data following the introduction of the AneVivo device into clinical practice indicate that this approach may provide an effective alternative to current in-vitro techniques for generating embryos in the IVF setting. However, larger properly powered studies will be required to confirm these initial findings.

SP3B.4 Pioneering invasive cell types of in vitro implanting human embryos revealed by combined morphologic and gene expression analysis

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Aims & objectives: Unravelling the mechanism of human embryo implantation is necessary to improve ART outcomes. In vitro models allow for the interrogation of dynamic interactions between embryonic and endometrial cells. Here we

characterise human embryo implantation into the Ishikawa endometrial epithelial cell line by combining morphologic and gene expression analyses.

Methods: Live and fixed human embryo-Ishikawa cell co-cultures were examined by high resolution fluorescence microscopy using cell structure-specific dyes and antibody markers. Matched analysis of embryo gene expression was afforded through RT-qPCR.

Results: Hatched day 6 human blastocysts attach rapidly (15/20 attached after 6h), and go on to invade the Ishikawa cell layer (37/46 invading after 48h). Immunofluorescence revealed that all invasive embryos contained multinucleated syncytiotrophoblast (ST), and that most invading blastocysts exhibited a collapsed blastocoel ($p < 0.01$). Moreover, we show for the first time that ST initiates the breaching of epithelial endometrial epithelial cells ($n = 7$). Utilising fluorescent dyes for live imaging allowed us to morphologically characterise this process before purifying RNA for gene expression analysis. From a panel of 19 trophoblast-related genes, 8 were found to be specific to day 6-8 human blastocysts and not expressed in Ishikawa cells ($n = 12$). Of these, GCM1, DLX3, HTRA4, GATA3, ERVW1 and PGF were consistently more highly expressed in embryos exhibiting invasive ST. Moreover, members of the highly homologous pregnancy specific $\beta 1$ -glycoprotein family (PSG1, 2, 3, 5, 6, 7, 8, 11) were expressed in day 8 embryos implanting into Ishikawa cells, but not in control embryos maintained in culture without Ishikawa cells, and were elevated in ST-positive invasive embryos.

Conclusions: Data from our in vitro model therefore implicate ST formation as the key event initiating human embryo implantation, and highlight potential markers of early human embryo invasion which may lead to clinical applications as well as new biology.

Short papers session 3C: Sperm

SP3C.1 Rapid 'switching' of human sperm motility

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Motility of human sperm is typically quantified as a 'snapshot', estimating the proportion of cells showing each motility type. However, observation of cells for several seconds suggests that behaviour of individual sperm can change rapidly (2). Such behavioural switching may be adaptive, for instance during ascent of the female tract by 'hopping'. We captured behaviour of individual sperm over a period of 180 s (9000 frames at 50 Hz), using a motorized stage to centre the cell in the field of view when required.

For analysis 4 behaviours were defined. Types 1-3 resembled activated, transitional and hyperactivated behaviours. Type 3 cells occasionally arrested with the anterior flagellum in a 'J' shape (type 4). Each of 180, 1 s periods were assigned to one of these behaviours. A subset of cells was also analysed using Metamorph software to generate continuous 3 minute tracks. Fractal dimension analysis (1) confirmed that visual analysis reliably identified types and changes of behaviour. % hyperactivation under each incubation condition was separately assessed by CASA.

In control recordings (EBSS pH 7.4) 16/18 cells showed repeated, abrupt transitions in behaviour (mean = 6.4 ± 0.8 min⁻¹, $n = 18$). Under conditions that raise $[Ca^{2+}]_i$ and stimulate hyperactivated motility (2 mM 4-aminopyridine at pH 8.5, hyperactivation increased from $4 \pm 2\%$ to $35 \pm 4\%$), switching between behaviours persisted (9/20 cells switched within 180 s) but the duration of periods of type 3/4 (hyperactivated-like) behaviour from 5.9 ± 0.5 seconds (control) to 82.3 ± 11.2 seconds ($P < 5 \times 10^{-8}$). Duration of type 1 (activated-like) behaviour was little affected (12.9 ± 1.7 and 8.8 ± 2.7 seconds respectively; $P = 0.2$). We conclude that behavioural switching occurs continuously and that stimuli that induce hyperactivation greatly prolong periods of hyperactivated behaviour.

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SP3C.2 $[Ca^{2+}]_i$ oscillations regulate behaviour of free-swimming human sperm

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In immobilised human sperm, agonist-induced $[Ca^{2+}]_i$ elevation induces the generation of large $[Ca^{2+}]_i$ oscillations, which may play a role in regulating motility and AR (1,2). Whether such oscillations occur in free-swimming cells is unknown. We have used time-lapse, fluorescence imaging of free-swimming human sperm to investigate (a) whether $[Ca^{2+}]_i$ oscillations occur under these conditions and (b) their effects on motility of the cell.

Time series (3 Hz or 10 Hz; 100-300 s) were collected from 92 progesterone-stimulated, free-swimming, fluo4-labelled human sperm (chamber depth 20 μ m; 25 or 31°C). The cell was periodically re-centred in the field of view as required. Cells

were tracked using Metamorph software and analysed for fluorescence intensity ($[Ca^{2+}]_i$). Continuous sperm tracks were generated from X-Y coordinates so that sperm behaviour could be related to changes in $[Ca^{2+}]_i$. Large $[Ca^{2+}]_i$ oscillations ($\approx 150\%$ fluorescence increase; $\approx 60-90$ s period) were observed in 25-30% of cells analysed.

Oscillations typically had a symmetrical shape rather than the fast rise-slow decay seen in immobilised cells. The low frame acquisition rate precluded accurate CASA assessment of hyperactivation but average path velocity (VAP), straightness (STR) and fractal dimension (high in hyperactivated cells; 3), were calculated. Most cells clearly accelerated during periods of increased $[Ca^{2+}]_i$ and several also showed reduced STR (increased turning). In two cells $[Ca^{2+}]_i$ transients were clearly correlated with large increases in fractal dimension.

We conclude that $[Ca^{2+}]_i$ elevation, including oscillations, occurs in free-swimming cells, though the kinetics of these oscillation differ from those observed in substrate-attached cells. We propose that this $[Ca^{2+}]_i$ signalling activity regulates cell behaviour, potentially enabling escape of cells attached to the oviduct wall and/or generating periodic turning.

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SP3C.3 The intracellular actions of trequinsin improves sperm cell hyperactivation and viscous medium penetration

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Background & purpose: Asthenozoospermia is a leading cause of male infertility. ICSI is used as the primary treatment for this due to a lack of knowledge regarding the complex signalling mechanism that regulate motility. However, CatSper is recognised as a key regulator of motility through its control of extracellular Ca^{2+} influx and represents a plausible target for the development of potential therapeutic compounds due to its confined expression in sperm. Thus, the aim of this study is to identify compounds that increase $[Ca^{2+}]_i$ via CatSper in order to improve sperm cell function.

Experimental approach:

Phase 1 - Utilise High Throughput Screening (HTS) to identify compounds that are efficacious inducers of $[Ca^{2+}]_i$.

Phase 2 - Determine the effect on motility, viscous medium penetration and acrosome reaction.

Phase 3 - Determine the mechanism(s) of action through patch-clamp electrophysiology, HPLC and Fluorimetric assays.

Phases 2 & 3 conducted on human donor and patient cell populations.

Key results: HTS identified Trequinsin Hydrochloride, a putative PDE3 inhibitor. Examination of the pharmacological profile showed robust increases in $[Ca^{2+}]_i$ via modulation of CatSper ion channel directly ($p < 0.01$) and partially blocks K_{Sper} with no effect on pHi. Trequinsin also significantly increased cyclic- GMP ($p < 0.05$). Functionally Trequinsin increased cell hyperactivation and penetration into viscous medium in all donors tested and did not induce premature acrosome reaction. Of the 29 patients assessed, 90% responded significantly to trequinsin treatment with boosts in cell hyperactivation.

Conclusion: Extensive examination of trequinsin hydrochloride has shown novel pharmacological actions that stimulate cell hyperactivation and viscous medium penetration. HTS is effective at identifying potential novel therapeutics that act by elevating $[Ca^{2+}]_i$. Utilising novel therapeutics that act on CatSper could provide insight into the intracellular regulation of normal and impaired sperm and aid treatment options for patients.

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SP3C.4 The presence of spermatozoa alters the physical characteristics and microRNA content of extracellular vesicles secreted by porcine oviductal epithelial cells in vitro

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It is widely accepted that extracellular vesicles (EVs) are potent vehicles for intercellular communication in the female reproductive tract, potentially via their transport of microRNAs (miRNAs) (Fazeli, 2016). We have demonstrated that EVs secreted by porcine oviductal epithelial cells (POECs) contain miRNAs (Jamaludin, 2016) and more recently, that spermatozoa contribute EVs, which interact with POECs in co-culture (Konstantinidi, 2017). This study investigated how the physical

characteristics and miRNA content of EVs secreted by POECs alter in the presence/absence of spermatozoa. Porcine oviducts were collected and POECs harvested by collagenase digestion.

Boar spermatozoa were Percoll washed and diluted in serum-free M199. Experimental groups (POECs-only, spermatozoa-only, POECs plus spermatozoa) were cultured in EV-depleted M199 for 24 hours at 39°C. EVs were isolated from conditioned media using size exclusion chromatography and EV size, concentration and zeta potential evaluated by nanoparticle tracking analysis. EV miRNA was extracted, sequenced and bioinformatics analysis performed. POECs secreted EVs 143.04 (± 0.17) nm in size with a mean zeta potential of -25.69 (± 0.8) mV. Spermatozoa produced a distinct population of EVs determined by size (165.38 $\pm$ 4.73 nm) and zeta potential (-22.80 $\pm$ 0.56 mV). Conditioned media from POECs co-cultured with spermatozoa had no significant increase in total EV number compared to POECs-only. However, the larger sized spermatozoa-EVs were completely diminished in sperm-POEC co-cultures, indicating a net movement of EVs between spermatozoa and POECs.

Eighty-one EV-mediated miRNAs were detected by sequence analysis. A number of miRNAs were unique to an experimental group, POEC-only (13 miRNAs), spermatozoa-only (1 miRNA), POEC plus spermatozoa (5 miRNAs). Bioinformatics analysis suggests that these miRNAs are involved in innate immune responses, cell proliferation and cellular migration. Clarification of the role of EVs in transporting miRNAs, and their influence on spermatozoa-oviductal communication will help us to devise novel diagnostic tools and therapeutic approaches to treat infertility.

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Short papers session 3D: Emerging reproductive technology and applications

SP3D.1 Programming prostaglandin and oxytocin receptor signalling in the human uterus: Mechanisms and applications

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In the human pregnant myometrium the prostaglandin E2 receptor, EP2, is a dually coupled G protein coupled receptor (G α s and G α q/11), enabling it to mediate both myometrial quiescence (anti-labour) and pro-inflammatory pathways (pro-labour)^[1]. Following the onset of labour, EP2 signalling is reprogrammed to favour the pro-inflammatory pathway via unknown mechanisms. Oxytocin receptor (OTR) signalling via G α i/o and G α q/11 is critical for labour and may be crucial for altering EP2 signalling at the onset of labour.

Myometrial biopsies were taken from term pregnant women undergoing caesarean section before or after the onset of labour. Using an EP2-specific agonist, butaprost, cAMP signalling was significantly decreased following the onset of labour with an enhanced ability to increase levels of pro-labour/pro-inflammatory pathways, COX-2 and IL-6. These EP2-mediated pathways, however, became sensitive to G α i inhibitor pertussis toxin (PTX) in samples taken following the onset of labour. The role of OT/OTR in mediating these alterations in G protein signalling was confirmed by in vitro pre-treatments of OT. To determine if OTR and EP2 mediate these events via direct associations, their ability to dimerise was measured using bioluminescence energy transfer and super-resolution photo-activated localisation microscopy. This revealed constitutive association between EP2 and OTR that underwent significant decreases in heterodimers and heterotrimers (p<0.05, p<0.01 respectively) following treatment with OT. Furthermore, the organisation of individual heterotrimers, tetramers and pentamers was altered by OT.

These results demonstrate that in vivo exposure to oxytocin reprograms EP2 signal activity, enhancing pro-inflammatory pathways via G α i/o pathways, instead of G α q/11 pathways, to elicit an inflammatory, pro-labour response. OTR-EP2 heteromers are constitutive yet modified with OT treatment, resulting in formation of multiple distinct signalling complexes to reprogram prostaglandin EP2 signalling during labour and offering the potential for novel therapeutic strategies for pre-term or post-term labour management.

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SP3D.2 Isolation of putative human oogonial stem cells by a NANOG SmartFlare RNA detection probe and fluorescence-activated cell sorting

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Background: Controversy has surrounded the isolation of putative oogonial stem cells (OSCs) from the adult mammalian ovary using antibody-based fluorescence activated cell sorting (AB-FACS) to the germ line marker DDX4^[1,2]. In this study we

report a novel isolation method using a SmartFlare RNA detection probe specific for the pluripotency maintenance factor NANOG. The Smartflare 'capture' oligonucleotide binds to target RNA releasing a fluorescent 'reporter' oligonucleotide, detecting NANOG-positive cells. NANOG is present in primordial germ cells and in putative OSCs isolated by DDX4^[3] making it an appropriate candidate for OSC isolation.

Aims: Our aims were 1) to isolate and characterise NANOG-positive cells and 2) to determine the degree of overlap between ovarian cells labelled with SmartFlare and DDX4. Methods Human ovarian cortical biopsies were obtained from 20 women aged 23-41 at elective caesarean section. Dissociated tissue was incubated with SmartFlare for 16 hours, NANOG-positive and negative cells were then FACS sorted. After dual-labelling with DDX4, cells were sorted into 4 groups: 1) NANOG+ only, 2) NANOG+/DDX4+, 3) DDX4+ only and 4) double negative cells. Sorted cells were cultured to assess their proliferative potential or collected for RT-PCR.

Results: NANOG+ cells expressed stem cell and germline markers. Dual-labelling identified a sub-population of NANOG+ cells that did not express DDX4. NANOG+/DDX4+ cells showed a similar molecular profile to previously reported DDX4-positive putative OSCs³. During 26 days of culture NANOG+/DDX4- cells showed expression of DDX4, indicating cell differentiation *in vitro*.

Conclusions: We have identified a novel isolation method for putative OSCs based on detection of NANOG RNA. This methodology removes reliance on a single factor to isolate putative OSCs, as dual-labelled FACS allows the identification of cells expressing NANOG with or without DDX4. Initial characterisation suggests the potential for NANOG+ cells to initiate DDX4 expression *in vitro*, a key step in normal germ cell development.

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SP3D.3 Fucosylation of Immunoglobulin G in the peripartum period is associated with the subsequent development of endometritis in dairy cattle

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Endometritis is a significant inflammatory condition of the uterus resulting in reduced fertility. Immune alterations during pregnancy and the reversion to normality across the peripartum period are important for postpartum uterine health. Immunoglobulin G (IgG) is an important immune mediator of pathogen defence and during pregnancy; the immune actions of IgG are modulated by glycosylation and this is reversed after birth. Differences in IgG glycosylation are associated with chronic inflammatory disease. Therefore, the aim of this study was to identify whether differences in the glycan structure of IgG in postpartum cows are associated with the development of endometritis.

In study 1, blood samples were collected from 96 dairy cattle approximately 10 days before calving and at 7, 14 and 21 days postpartum (dpp). In study 2, blood was collected from 122 dairy cattle at 7dpp. Uterine health was monitored by vaginal mucus assessment and animals were retrospectively diagnosed as healthy (HTY) or as having developed endometritis (ENDO) at 21 dpp based on standard disease definitions¹. IgG was purified and the glycan fraction of the immunoglobulin was released before being quantified by ultra-performance liquid chromatography.

Thirty-one glycan peaks representing different glycan structures were identified in bovine IgG. The percentage of IgG fucosylation, as identified by higher quantity of fucosylated glycans, was increased in ENDO vs HTY cows. In study 1, IgG fucosylation in ENDO cows was significantly higher on day 10 pre-calving and on 7, 14 and 21 dpp (P<0.001). In study 2, IgG fucosylation was significantly higher on day 7pp in ENDO cows vs HTY cows (P<0.001). During the peripartum period, fucosylation of IgG is increased in dairy cows that go on to develop endometritis. This suggests that optimum regulation of peripartum immunity, in particular antibody glycosylation, may be important for maintaining uterine health following birth. All procedures were carried out under authorisation of the Irish Department of Health and Children and approved by the University College Dublin Animal Research Ethics Committee (AREC-P-10-53 and AREC-14-08-Williams). The work was funded by Science Foundation Ireland (07/SRC/B1156) and the Irish Department of Agriculture, Food and the Marine (13-S-472). 1. Sheldon et al. (2006) Defining postpartum uterine disease in cattle. *Theriogenology* 65; 1516-1630.

Short papers session 3E: Pregnancy outcomes

SP3E.1 Fertility treatment with donor eggs: The influence of legal and technological evolutions on patients' behaviour

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Background: As women continue to delay child bearing, egg donation is increasing in importance. However, sourcing donor eggs remains a challenge. The changing regulatory landscape and advances in technology are likely to be impacting on the characteristics of egg donors as well as the way in which eggs are utilised. In this study we report how these changes have altered our practice over the past 13 years.

Methods: This retrospective study reviews over 2000 treatment cycles using donor eggs from 2005 to 2018. The study focuses on the donor egg sources, frozen egg use, demographics of patients receiving treatment with donor eggs and pregnancy outcomes.

Results: The 2012 legal changes in United Kingdom regarding egg donation and the creation of a local egg bank reversed the previous trend to seek cross border treatment for egg recipients. Since 2014, all our recipients have been treated with donor eggs from the local bank. Moreover, the egg sharing programme providing eggs to 17% of the recipients in 2013 dropped to one case in 2018. The demand for treatment with the donor eggs appears stable among our patient population. Despite the permissive local legislation regarding the age limit for fertility treatment, the demand for treatment for recipients over 50 years old remains limited. The most striking change has been the growth in the use of frozen eggs. Comparable pregnancy outcomes are now achieved using frozen or fresh oocytes, and this has led to 95% of recipients now receiving treatment with frozen eggs compared with just 1% in 2013.

Conclusions: Although limited to one centre, this descriptive study illustrates how legal and technological changes have resulted in remarkable change in the sourcing and profile of donors, and the way in which their eggs are being utilised in clinical practice.

SP3E.2 UK surrogacy law - what does the future hold?

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In *Re Z (A Child) (No 2)*, the President of the Family Division, Sir James Munby, decided that certain provisions of the Human Fertilisation and Embryology Act are incompatible with the European Convention on Human Rights because only couples (rather than single parents) may seek a declaration of legal parenthood for a child born through surrogacy. This is just one illustration of how existing UK surrogacy laws are woefully inadequate to meet society's needs in this day and age. This case finally put surrogacy law reform on the political agenda, with the government confirming their support for change.

This talk will consider the main controversies and difficulties which commissioning parents face under current UK surrogacy laws, and what is being done by way of reform to address these. The key areas of the current law which most require reform will be discussed, with reference to a variety of controversial caselaw in this area. Details of the proposed changes and improvements will then be explored. The presentation will look at what is being done to enable changes to the law to be made, including the Law Commissions' consultation, as well as the establishment of an APPG to review the law and promote debate. The presentation will conclude with a comparison of current UK law with international law, including those countries where the laws are particularly lenient or strict, and alternative models of regulation.

SP3E.3 Vitamin D and assisted reproductive treatment outcome: A systematic review and meta-analysis

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Study question: Is serum vitamin D associated with live birth rates in women undergoing ART?

Summary answer: Women undergoing ART who are replete in vitamin D have a higher live birth rate than women who are vitamin D deficient or insufficient.

What is already known: Vitamin D deficiency has been associated with an increased risk of abnormal pregnancy implantation as well as obstetric complications such as pre-eclampsia and fetal growth restriction. However, the effect of vitamin D on conception and early pregnancy outcomes in couples undergoing ART is poorly understood.

Study design, size, duration: A systematic review and meta-analysis of 11 published cohort studies (including 2476 women) investigating the association between vitamin D and ART outcomes.

Participants/materials, settings & methods: Literature searches were conducted to retrieve studies which reported on the association between vitamin D and ART outcomes. Databases searched included MEDLINE, EMBASE, Cochrane Central Register of Controlled Trials and CINAHL. Eleven studies matched the inclusion criteria.

Main results and the role of chance: Live birth was reported in seven of the included studies (including 1897 patients). Live birth was found to be more likely in women replete in vitamin D when compared to women with deficient or insufficient vitamin D status (OR 1.34 [1.06--1.70]). Five studies (including 1571 patients) found that women replete in vitamin D were more likely to achieve a positive pregnancy test than women deficient or insufficient in vitamin D (OR 1.31 ([0.97--1.76])). All 11 of the included studies (including 2476 patients) reported clinical pregnancy as an outcome. Clinical pregnancy was found to be more likely in women replete in vitamin D (OR 1.47 [1.04--2.10]). Six studies (including 1506 patients) reported miscarriage by vitamin D concentrations. There was no association found between miscarriage and vitamin D concentrations (OR 1.24 [0.84--1.82]). The included studies scored well.

SP3E.4 Is a woman's chronological or 'ovarian' age more important in determining perinatal outcome after ART?

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Background: Although ovarian reserve (OR) decreases as women age, the rate at which this occurs varies. Older women are at increased risk of complications such as miscarriage, preterm labour and fetal growth restriction during pregnancy, but it is unclear whether premature ovarian ageing is associated with suboptimal perinatal outcomes. This information would help in counselling women with poor OR embarking on IVF treatment.

Objectives: To determine whether poor OR influences perinatal outcomes independent of age.

Methods: We retrospectively reviewed all fresh IVF/ICSI cycles in which a single embryo was transferred between 1/1/10 and 31/12/16. Poor OR was defined as an AMH ≤ 5.4 pmol/l whilst between 5.41 and 24.99 pmol/l was considered normal. We collected data regarding cycle outcome and, where applicable, information concerning fetal anomalies, gestational age at delivery and birth weight.

Results: We identified 1520 women, of whom 1197 (78.8%) had normal OR and the remaining 323 had poor OR. The mean ages of women with normal OR was 35.3 ± 4.3 years and poor OR was 36.9 ± 3.8 years ($p < 0.0001$). Following treatment, 705 (46.4%) women had a positive pregnancy test. Once pregnant, after adjusting for maternal age, women with poor OR ($n=109$) were no more likely to experience a biochemical pregnancy or miscarriage (41.3% versus 41.6%, aOR 1.1, 95%CI 0.7-1.6, $p=0.809$) than women with normal OR ($n=596$). There were no significant differences in rates of fetal anomalies between women with poor and normal OR (1.8% versus 1.2%, $p=0.636$). Furthermore, there were no significant differences in birth weight (3272 ± 630.7 g versus 3376.4 ± 576.3 g, aMD 105.5g, 95%CI -62.0-273.0, $p=0.216$) or gestational age at delivery (38.9 ± 2.3 weeks versus 39.1 ± 2.1 weeks, aMD 0.3 weeks, 95%CI -0.5-1.0, $p=0.517$) between women with normal or poor OR.

Conclusion: Our data suggests that once pregnant, OR does not appear to affect pregnancy loss rates, incidence of fetal anomalies, birth weight or gestational age at delivery after adjusting for maternal age.

Sperm

P001 Percutaneous epididymal sperm aspiration (PESA) in obstructive azoospermia: Comparing cryopreserved versus fresh PESA sperm

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Background: Sperm can be retrieved surgically from epididymides or testicles to assist fertility treatments for couples where the male partner suffers from obstructive azoospermia. Sperm can be used immediately or cryopreserved for future fertility treatment, allowing men to father their own genetic offspring through ICSI, where otherwise they could only use donor sperm or adopt. We offer cryopreservation for future use. The aim of this audit was to compare cryopreserved PESA success rates to published figures in the literature for cryopreserved and fresh PESA.

Methods: Retrospective audit, July 2013 - October 2017, men undergoing PESA in local fertility unit, looking at cause and method for surgical sperm retrieval.

Results: The number of patients included were 25 with the main indication for PESA being previous vasectomy (100%); 52% had failed reversals. PESA successful (92%) with 80% suitable for cryopreservation and 76% suitable for ICSI. Local versus (*Published literature figures*): PESA fertilisation rate, 53% (53% cryopreserved, 56% fresh); blastocyst formation rate, 45%. Clinical pregnancy rate, 33% (37% cryopreserved, 37% fresh); live birth rate, 31% (26% cryopreserved, 27% fresh) and twin pregnancy rate, 11% (*no published figures*) 1,2 . 36% couples had no associated female factors, 32% mild and 8% severe female factors. Significant female factors resulted in lower local clinical pregnancy (11%) and live birth (10%) rates.

Conclusions: Our outcome for primary use cryopreserved PESA sperm, compares favourably with published data in all parameters. Consistent with previous views, female factors do have an impact on clinical pregnancy and live birth rates when male factor infertility secondary to vasectomy is the main cause of infertility. Our data demonstrates that cryopreserved PESA sperm can be used routinely for fertility treatment with similar success rates to fresh PESA sperm allowing more flexibility in timing of ICSI treatment, consistent with published data.

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P002 Correlation between human sperm parameters (concentration, progressive motility, velocity) and microRNA expression pattern following cryopreservation

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Study question: Observe the differential expression of miRNAs in human spermatozoa frozen with two different cryoprotectants (CPA), as well as correlate their expression levels with semen parameters.

Summary answer: Increase in the level of miRNA expression in human spermatozoa frozen with CPA containing test-yolk buffer. Overall, some level of correlations with semen parameters (Concentration, PM% and velocity). What is known already: Sperm cryopreservation has been suggested as a novel approach to preserving fertility in men undergoing treatment for infertility or cancer or vasectomy. MiRNAs are small non-coding single stranded RNA structures with an average size of 22 nucleotides, which have been shown to regulate cell proliferation, differentiation, and apoptosis. It has been reported by many studies that miRNAs affect spermatogenesis at different stages. However, there is a limited number of studies, if none, looking at the correlation between sperm parameters and possible alterations in miRNA expression levels following cryopreservation using different cryoprotectants (CPAs).

Study design, size, duration: The semen samples of 16 healthy donors were collected over a 4-week period to be ultimately used for freezing-thawing using two different CPAs and extracting of target miRNAs (miR-24, 193b, 320A, 34C, Let-7b and 18S). Participants/materials, setting, method: Semen samples frozen via controlled rate freezing using Test-Yolk buffer (TYB) and Quinns advantage sperm freezing media (SAGE). After thawing, concentration, PM% and velocity analysed via computer-assisted sperm analysis (CASA). Samples subjected to miRNA extraction via Qiagen miRNeasy Mini Kit. Real-time PCR reactions performed in triplicates using 7500 Fast Real Time PCR System. MiRNA expression level (24, 193b, 320A, Let 7b and 34C) measured using the threshold cycle method using 18S results as internal control.

Main results and the role of chance: MiRNA expression pattern increased in spermatozoa frozen with CPA containing test-yolk buffer, however the difference was not statistically significant. MiR-193b remained undetected in all frozen semen samples (TYB and SAGE). MiR-24 and miR-34C exhibited negative relationship with the concentration whereas miR-320A and miR-Let 7b demonstrated positive association in case of spermatozoa frozen with TYB. Nonetheless, miR-24, 320A and 34C showed stronger positive and miR-Let 7 b showed negative association with concentration for SAGE. In case of PM%, miR-24 and miR-Let 7b demonstrated negative association for TYB, however, miR-24, Let 7b and 34C had a positive relationship for

SAGE. Mir-24 and miR-320A demonstrated positive and miR-24, 320A, Let 7b and 34C showed negative relationship with velocity for TYB and SAGE respectively. Nevertheless, all of the correlation was found statistically non-significant.

Limitations, reason for caution: Some of the limitations included: high inter-donor variations in terms of semen parameters as well as small sample size. The removal of the supernatant after washing could differ between samples.

Wider implications of the findings: The results of the study revealed some level of correlation between miRNA expression profiles and semen parameters, as well as the effect of cryoprotectants on miRNA expression.

Study funding/competing interest(s): The work was supported by The University of Nottingham.
Trial registration number: N/A

P003 An evaluation of the characteristics of azoospermic men who had successful sperm retrieval and those who did not using Trucut needle testicular sperm extraction

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Purpose: This study compares the characteristics of two groups of azoospermic men: those who had sperm successfully retrieved (positive biopsy), and those who did not (negative biopsy), at Trucut needle testicular sperm extraction (TESE).

Method: This retrospective observational study was undertaken in a tertiary referral NHS fertility unit. Medical records and information systems were interrogated to obtain data. Sixty-seven negative biopsy subjects were identified and compared to 98 positive biopsy subjects. Data was analysed using z-test, chi-square or Fisher's exact tests.

Results: Those with negative biopsies were significantly younger (32.5 yrs vs 38.1yrs; z-score 5.76; p-value<0.0005). Men with positive biopsies were more likely to have fathered children (39(41%) vs 1(1.4%); χ^2 33.04; p<0.0005).

In the positive biopsy group, it was significantly more likely that a condition in the patient's history such as cystic fibrosis or previous vasectomy could account for the azoospermia (z-score 2.57; p-value 0.009).

Reduced testicular volume was observed in 59.4% in men with a negative biopsy compared to 11.4% with a positive biopsy (z-score 5.769 p-value <0.0005). Serum FSH levels were higher in those with negative biopsies (12.27 vs 7.23; z-score 5.123; p<0.0005), as were serum LH levels (4.67 vs 5.98; z-score 2.192; p=0.03). Serum testosterone levels were significantly lower (14.332 vs 16.24; z-score 2.018; p-value 0.04).

In negative biopsies, 7(22.6%) of those tested had an AZF gene deletion compared to none in the positive biopsies (p-value 0.001). There was a higher rate of cystic fibrosis in the positive biopsy group (21/96(21.9%) vs 3/66(4.5%); p-value 0.0028). There was no significant difference in karyotype abnormalities - positive biopsy group 1/42(2.38%) vs 1/44(2.27%); p-value 0.97.

Conclusion: Both groups of patients are quite defined with differences reaching statistical significance apart from karyotype. Having knowledge of the characteristics of these groups can guide us to better manage patient expectations.

P004 A comparison of ICSI outcomes using fresh testicular sperm and frozen-thawed testicular sperm from azoospermic men who underwent Trucut Needle Testicular Sperm Extraction

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Purpose: To compare the outcomes of ICSI treatment using fresh testicular sperm (FTS) or frozen-thawed testicular sperm (FTTS) from azoospermic men who underwent Trucut needle testicular sperm extraction (TESE).

Method: A retrospective observational study undertaken in a tertiary referral NHS fertility unit. Medical information systems were interrogated for data. Outcomes of TESEs (n=146) between 2014-17 were obtained. The data was analysed using as appropriate Z-test, Chi-squared or Fisher's exact test. P-value of 0.05 was used to determine significance throughout.

Results: There were 66 FTS and 80 FTTS biopsies. There was no significant difference between the average age of the subjects (FTS 37.68yrs vs FTTS 38.78yrs (p 0.33); average age of partners (FTS 33.1 vs FTTS 33.88 (p 0.35); partners' AMH levels (FTS 24.27 vs FTTS 25.89 (p 0.62); the number of oocytes collected (FTP 718 vs FTTP700 (p 1.30) and the numbers of oocytes injected (p 0.02).

Significantly more embryos were produced with FTS (397 embryos from 635 oocytes) compared to FTTS (303 embryos from 604 oocytes; χ^2 23.44, p 0.0001). Significant differences were observed in pregnancy rate (FTS 55% vs FTTS 36% of cycles; χ^2 5.3; p 0.02) and live birth rate (FTP 42.5% vs FTTS 24.24% of cycles; χ^2 5.6; p 0.017).

No significant difference was observed between day of transfer (Day 3/5) and success rates (24 (32%) vs 19 (38.6%) χ^2 0.22; p 0.09). Whilst there were positive trends towards increased success with embryos fertilised using FTS, there was no significant difference in outcome for fresh/frozen Day 3/5 Single/Double embryo transfers (χ^2 6.98; p0.64).

Conclusion: Live birth rates, pregnancy rates and fertilisation rates were significantly better using FTS than FTTS. The retrospective design is an acknowledged limitation of this study.

P005 Advancing sperm cryopreservation: Does pre-freeze sperm preparation confer any advantage over the traditional post-freeze approach?

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Introduction: Human sperm cryopreservation is characterised to this day by suboptimal success rates. Interestingly, a traditional approach to improving post-thaw outcome has been to integrate standard sperm preparation techniques into freezing protocols as a means of selecting sperm with the highest fertilisation potential prior to insemination. However, no consensus has been reached yet regarding the optimal timing (pre-freeze vs post-freeze) of this selection step. As such, the aim of the present study was to investigate whether pre-freeze sperm preparation by density gradient centrifugation (DGC) would improve the cryopreservation outcome of human semen samples when compared to post-freeze DGC.

Methods: A total of 20 donor human semen samples were collected and divided into two aliquots. One aliquot was prepared by PureSperm DGC before being frozen by controlled rate freezing (CRF), while the other aliquot was prepared by PureSperm DGC after freezing by CRF. Fresh and thawed samples underwent computer assisted semen analysis (CASA) for the measurement of sperm motility, concentration and average motile speed, as well as eosin-nigrosin staining for vitality assessment. Finally, collected CASA video loops were used for the quantification of progressively motile spermatozoa exhibiting cryopreservation-induced coiled tail morphology.

Results: Higher post-thaw total ($p < 0.0001$), progressively motile ($p = 0.0005$) and vital ($p < 0.0001$) sperm counts were reported for semen samples when frozen after DGC preparation. Conversely, lower counts of progressively motile spermatozoa with coiled tails ($p = 0.015$) were observed in post-thaw semen samples when freezing was performed prior to DGC preparation. Finally, the timing of DGC had no significant effect on post-thaw sperm average motile speed.

Conclusion: The present study suggests that direct insemination with frozen-prepared sperm with minimal intervening post-thaw processing might be a more advantageous approach to current clinical practices, particularly for donor and patient intrauterine insemination programmes. Further research into the cryopreservation-induced coiled sperm tail morphology is also warranted.

P006 Is SpermMobil the "Magic Potion" for 100% immotile sperm samples?

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Purpose: To determine the effect of SpermMobil (Gynemed) on 100% immotile samples and whether its use leads to positive clinical outcomes. Traditionally, viable sperm are selected on their ability to move. The injection of immotile sperm leads to reduced fertilisation rates, likely as a result of the selected sperm being non-viable². SpermMobil contains Theophylline, a molecule that increases sperm energy by blocking phosphodiesterase activity¹. Its addition should stimulate movement if the sperm are viable.

Method: This was a retrospective analysis of clinical outcomes for patients treated during April 2016 to July 2018, who had 100% immotile sperm before the addition of SpermMobil ($n = 15$). Samples were fresh or frozen and included ejaculated ($n = 3$) and surgically retrieved sperm ($n = 12$). SpermMobil was added to prepared sperm samples 10 minutes before sperm selection for ICSI was attempted.

Results: Following the addition of SpermMobil, only 1 patient's sperm remained completely immotile. ICSI was undertaken for the remaining 14 patients, all of whom achieved fertilisation, with overall fertilisation rate of 54% (79/146). Ten women underwent fresh embryo transfer resulting in 8 clinical pregnancies (80%). Three patients had elective freeze with subsequent FET, 2 achieved a clinical pregnancy (67%). Only 1 patient had no embryo transfer (due to embryo arrest at morula stage). Overall, of the 15 patients in this study, 10 (67%) achieved a clinical pregnancy following use of SpermMobil.

Conclusions: The addition of SpermMobil to 100% immotile sperm samples aids selection of viable sperm. Its use leads to successful fertilisation, blastocyst formation and positive clinical outcomes with good clinical pregnancy rates in both fresh and frozen cycles. SpermMobil is a valuable tool as the stimulatory effect reduces the time taken to find viable sperm. It helps prevent the injection of potentially non-viable immotile sperm.

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P007 Trialling use of MiOXSYS oxidative stress assay in a fertility centre

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Background: Oxidative stress mediated DNA damage contributes to male factor infertility^[1]. Oxidative stress also affects sperm function via membrane peroxidation^[2]. Various measures of oxidative stress and DNA damage have therefore been proposed as potentially insightful additions to conventional semen analysis^{[3][4]}. The current method used in our clinic to assess samples for DNA damage is SpermComet (Examen). This electrophoresis based method requires sending samples off site, delaying results. We trialled use of the MiOXSYS system (Aytu), a rapid inexpensive assay of oxidative stress, to ascertain if results are comparable with SpermComet.

Methods: Donor (n=15) and patient (n=46) samples were analysed using SpermComet (snapfrozen, analysed externally) and MiOXSYS (30µl of semen pipetted onto disposable sensor). SpermComet results reported as average Comet score of assessed sperm: high, average or low DNA damage, MiOXSYS results reported as static oxidation redox potential.

Results: SpermComet results were obtained for all samples within ten days. MiOXSYS results were not obtained for 5 samples (8%), others were available within ten minutes. 3 samples (5%) were reported to have high levels of DNA damage, these were all above the MiOXSYS normal threshold. An additional 6 samples (10%) had high MiOXSYS results, of which two had low Comet scores.

Conclusions and further work: Two distinct, related, parameters were assessed. Our results suggest samples with high levels of DNA damage have high oxidative stress, and are identified by either method. However for some samples, high oxidative stress was detected alongside medium or low levels of DNA damage. As oxidative stress can affect sperm independently of DNA damage, this may indicate suboptimal samples not identified by SpermComet. Initial MiOXSYS results are promising, and an inexpensive rapid in-house assay could be of use when assessing donor and patient samples, including for treatment. However further work is needed to test the significance of results, including comparison with ART outcomes.

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P008 TART tumors and its impact on male reproductive potential in Congenital adrenal Hyperplasia

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Introduction: CAH is an autosomal recessive condition which mainly affects adrenal steroidogenesis. It is most commonly caused by deficiency of 21 Hydroxylase enzyme. TART tumours are rarely the presenting symptoms and hence are not diagnosed till late. However, the late diagnosis and its management can have impact on fertility.

Clinical presentation: We present a case of an adult with childhood diagnosis of CAH presenting at a teaching university hospital. A 30 yr old male presented with primary sub fertility issue. He had background history of CAH - salt losing variant with early puberty and short stature and a strong family history with 5 additional family members known to have been affected with CAH. He was diagnosed with severe oligoasthenoteratozoospermia at the age of 23 at a different fertility unit. After having tried to achieve conception for more than 2 years, he had an ICSI cycle with poor sperm count which unfortunately did not achieve conception. On presentation at our university teaching hospital, on initial screening, he was found to have azoospermia. Apart from this, there were no additional presenting symptoms. He was investigated further in form of male hormonal assay and ultrasound testes as per the units protocol along with male karyotype. He had a normal 46XY karyotype with normal hormonal assay. The US testes showed a bilateral testicular adrenal rest tumours.

Conclusion: TART is common cause of sub fertility in CAH patients. It is often not detected early and can lead to significant impact on semen parameters. It is very important for all the clinicians to be aware of existence of such an entity which has a progressive course affecting the male reproductive potential. Early diagnosis and timely intervention either in form of freezing sperm sample or advising to have a family earlier in life is crucial.

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P009 Differences in glucose, fructose and pyruvate metabolism by human sperm recovered from normozoospermic and asthenozoospermic ejaculates

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Background: Whilst asthenozoospermia can be readily identified at semen analysis, the underlying causes are unclear. Sperm motility is dependent on energy production through glycolysis or oxidative phosphorylation, yielding lactate and bicarbonate respectively. Since Density Gradient Centrifugation can separate sperm into high (80% pellet) and low (40% interface) motility fractions, we hypothesised that metabolic differences between these sperm from normozoospermic and asthenozoospermic ejaculates may aid understanding of sperm energy production for motility.

Aim: Measure lactate and bicarbonate from metabolism of ^{13}C -labelled substrates by high and low motility sperm from normozoospermic and asthenozoospermic ejaculates by Magnetic Resonance Spectroscopy (MRS).

Materials and methods: Semen was obtained from 132 men attending diagnostic semen analysis (NHS Research Ethics Committee approval), of which 36 showed asthenozoospermia. Each sample was washed in 80/40% Percoll-PBS gradients. Antibiotics and either ^{13}C -glucose, ^{13}C -fructose or ^{13}C -pyruvate was added to 80% and 40% sperm before incubation at 37°C for 4 hours. ^{13}C spectra of the samples were acquired using a 9.4T MRS spectrometer. Lactate and bicarbonate integrals were normalised to live sperm concentration and compared by Kruskal-Wallis test.

Outcomes: For all substrates lactate was lower in 80% than 40% sperm of both normozoospermic and asthenozoospermic samples. Lactate from glucose was significantly lower for 80% sperm of normozoospermic ejaculates compared to 40% sperm of normozoospermic or asthenozoospermic samples ($p=0.009$, $p=0.03$, respectively). For pyruvate incubations, 80% sperm from asthenozoospermic ejaculates had significantly lower lactate than 40% sperm from both normozoospermic and asthenozoospermic ejaculates ($p=0.0006$, $p=0.01$), and lactate from 80% sperm was significantly lower than 40% sperm of normozoospermia samples. Fructose incubations showed no significant difference in lactate. Bicarbonate showed no significant differences.

Discussion: Higher lactate levels produced by 40% sperm may indicate that impaired motility of sperm is the result of their inability to regulate their energy metabolism compared to highly motile sperm.

P010 An analysis of endogenous metabolites in sperm from asthenozoospermic and normozoospermic ejaculates by ^1H Magnetic Resonance Spectroscopy

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Background: The underlying causes of asthenozoospermia are not well understood but Magnetic Resonance Spectroscopy (MRS) is a method that can profile the metabolites in sperm from different populations. The advantage of MRS is that the sperm remain viable throughout the measurement, potentially allowing further tests to be performed or be used in assisted reproduction.

Aim: Use spectrum binning of ^1H -MRS spectra to identify any differences in the endogenous metabolome of washed sperm from normozoospermic and asthenozoospermic ejaculates.

Materials and methods: Semen was obtained from 60 men attending for diagnostic semen analysis (NHS Research Ethics Committee approval), of which 15 showed asthenozoospermia. Each sample was washed in 80/40% Percoll-PBS gradients to recover high motility (80% pellet) and low motility (40% interface) sperm. ^1H spectra were acquired using a 9.4T MRS spectrometer at 37°C and these were binned at 0.04ppm, with the water peak (4.5-5.2ppm) removed. Binned spectrum integrals were normalised to sperm concentration and comparisons made using a two-way ANOVA with a Bonferroni multiple comparison test.

Outcomes: In the choline region (3.19 and 3.23 ppm) the integral for sperm recovered from the 80% fraction was significant lower for asthenozoospermic compared to normozoospermic ejaculates ($6.7 \pm 1.1 \times 10^7$ vs $8.2 \pm 0.8 \times 10^7$ respectively), both of which were significantly lower than for the corresponding sperm recovered from the 40% interface (asthenozoospermia: $11.3 \pm 2.1 \times 10^7$; normozoospermia: $11.6 \pm 1.0 \times 10^7$). In the lactate region (1.34, 1.30, 1.26ppm), integrals for 80% sperm (asthenozoospermia: $1.7 \pm 0.4 \times 10^7$; normozoospermia: $1.0 \pm 0.1 \times 10^7$) were significantly lower than both the 40% sperm (asthenozoospermia: $4.4 \pm 1.2 \times 10^7$; normozoospermia: $3.7 \pm 0.5 \times 10^7$), although in both cases these were not significantly different from each other.

Discussion: Choline and glycerophosphocholine are components in the sperm cellular membrane and differences shown here may reflect changes in sperm ultrastructure. Lactate, which is a by-product of energy metabolism, could show how high and low motility sperm have used endogenous substrates for energy production.

P014 Physical removal of bacteria as an alternative to antibiotics in boar semen extenders

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By law, antibiotics must be added to semen extenders when preparing commercial semen doses for artificial insemination, to counteract bacterial colonisation during semen collection and processing. However, such use could contribute to the development of antibiotic resistance. It was shown previously that centrifugation through a high density colloid can separate boar and stallion spermatozoa from bacteria in semen, as well as selecting functional spermatozoa from the rest of the ejaculate. However, some spermatozoa may be lost during selection, and recontamination of the sperm pellet may occur.

Objective: to determine whether a low density colloid can remove bacteria from boar semen samples and whether a modified tube to facilitate pellet retrieval can prevent bacterial re-contamination.

Methods: Ejaculates from 9 boars, extended in Beltsville Thawing Solution without antibiotics, were divided among the following treatment groups: control (C), colloid centrifugation (S), and modified colloid centrifugation (M). After SLC, all samples were cultured for bacterial content using standard microbiological methods. Sperm quality was checked daily.

Results: Three of the C and M samples and six of the S samples contained no bacteria. Mean bacterial counts for the remaining samples (colony forming units/mL) were: C 325±47; S 28±28; M 37±20. Using linear mixed-effects models with treatments and storage time as fixed effects, S and M contained significantly fewer bacteria than C ($P<0.01$); there was no difference in bacterial count between S and M. There were only marginal differences among treatments in sperm quality. **Conclusions:** centrifugation through a low density colloid can remove or reduce bacterial contamination in boar ejaculates without antibiotics. Furthermore, it is possible to collect boar semen without bacterial contamination by paying strict attention to hygiene. We thank the Society for Reproduction and Fertility for a pump grant and AIM Ibérica for the boar semen.

P015 Effects of Cisplatin exposure on immature human testis

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Background/objectives: Long-term survival rates of childhood cancer patients are now more than 80%, however, cancer treatment during childhood may result in infertility in adulthood. Cisplatin is one of the most commonly used drugs for childhood cancers but knowledge of the exact mechanism behind cisplatin-induced infertility is still limited. The human fetal testis is a model for the pre-pubertal testis as these tissues contain similar germ cell sub-populations. The present study aims to understand the effects of Cisplatin exposure on the immature human testis using this model.

Methods: Hanging drop cultures were set up using second trimester human fetal testes ($n=3-6$). Tissue was exposed to 0.5 µg/ml Cisplatin or control for 24hrs and kept in culture for a further 24, 72, 96 and 240 hours post-exposure. Germ cell numbers were evaluated by immunohistochemistry for gonocytes (AP2γ) and pre-spermatogonia (MAGE-A4) and manual quantification (+vely stained cells/tubular area (mm²)). Two-way ANOVA was performed to assess statistical significance.

Results: Cisplatin treatment caused no change in germ cell numbers after 24 hours. Longer exposure resulted in a significantly reduced number of gonocytes, thus at 72 hours: 277.2±65.1 vs 140.4±34.3 cells/mm² ($p=0.0001$), whereas the number of pre-spermatogonia was unchanged. In contrast, at 96 hours post-exposure to Cisplatin there was a significant reduction in pre-spermatogonia (298.1±99.5 vs 149.4±69.2 cells/mm², $p=0.0238$), with no effect on gonocyte number. At 240 hours, treatment with Cisplatin resulted in significant reduction in both gonocyte (363.4±89.0 vs 52.25±8.0 cells/mm², $p<0.0001$) and pre-spermatogonial (388.1±63.1 vs 169.7±33.1 cells/mm², $p=0.0002$).

Conclusions:

- Cisplatin exposure causes early loss of gonocytes in the immature human testis, whereas the effect on pre-spermatogonial number is delayed.
- Further studies are ongoing to assess proliferation and apoptosis in germ cells and to evaluate the effect of cisplatin exposure on somatic cell populations.

P016 Altered expression of testicular SCF and c-kit genes in antiretroviral therapy-induced semen alterations and subfertility

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Background: Antiretroviral therapy (ART) has been implicated in testicular toxicity observed in HIV patients. Optimal spermatogenesis has been shown to be dependent on the expression of Stem cell factor (SCF) and c-KIT genes. They are

known to be crucial for germ cell development and fertility. The study investigated the effects of ART and Naringenin on the expression of SCF and c-KIT genes in the testes of Sprague Dawley rats.

Methods: Thirty male rats weighing 200--220g, were randomly assigned into 6 treatment groups- DW: Distilled water, H: HAART, N40: Naringenin, 40 mg/kg, N80: Naringenin, 80 mg/kg, HN40: HAART+Naringenin, 40 mg/kg and HN80: HAART+Naringenin, 80 mg/kg. Treatment lasted for a period of 10 weeks. Copulation with adult non-mated females was allowed to take place. The number of pregnancies and pups were noted. Rats were sacrificed, testes were harvested and semen were analysed. Expressions of SCF and c-kit genes were done via real-time Polymerase Chain Reaction. The Animal Research Ethical Committee, UKZN, South Africa approved this research with a reference number AREC/046/016D.

Results: There was a significantly lower count in group H compared to DW and N40. There were significantly lower progressive sperms in group H when compared to DW, N40, N80, HN40 and HN80. The fertility index was higher in the DW than in the H and N40 groups. The number of pups per group were also higher. The animals in groups H, HN40 and HN80 displayed altered expression of SCF/c-KIT genes when compared to controls.

Conclusion: The study suggests that ART may alter the expression of SCF and c-KIT genes in the testes thereby causing deleterious effects on testicular function. Naringenin, a bioflavonoid may be a useful adjuvant therapy in protecting against testicular toxicity. Keywords: antiretroviral therapy, Stem Cell Factor, c-KIT, semen parameters, male infertility.

P017 Virtual reality visualization and simulation of spermatozoa-oviduct interactions as an outreach tool

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The oviductal environment poses a navigational challenge for spermatozoa involving a range of communication processes between the male gametes and the oviduct. Forming a clear picture of how spermatozoa interact with the oviductal environment, how the behaviour of different spermatozoa can affect this interaction and the success of the fertilization process, is challenging at best. To ease our understanding of such intricate biological processes, our group is using virtual reality (VR) technology to produce an immersive visualization of such complex scenarios. These visualizations can be used as a powerful educational tool for outreach purposes, educating students at different levels, as well as the general public. By overcoming the limitations imposed by 2D still images or videos, individuals get to experience the sperm transport process from a male gamete's point-of-view, this allows deeper conceptual understanding and ideally further familiarity with the ongoing interactions inside the oviduct. For people affected by infertility problems, these visualizations can foster awareness of the factors potentially contributing to their health problems.

Previous efforts in cardiology (1), psychology (2), andrology (3), and molecular dynamics (4), suggest VR is effective as a teaching and awareness bringing tool. However, producing biologically realistic data to inform the VR experience is no trivial task. To achieve a biologically-realistic visualization, we have reconstructed a set of virtual oviducts based on micro-CT (external morphology) and histology images (internal tissue folding) derived for mouse and pig oviducts and leveraged the simulated individual sperm paths generated by our previously developed predictive computational model of spermatozoa behaviour under a variety of conditions (5).

Thus, we have created a biologically grounded VR experience that can be used both to educate and predict the behaviour of the sperm-oviduct system.

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P018 The effect of sperm preparation technique between swim up with zeta method and density gradient centrifugation with zeta method

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Objectives: To compare the efficiency of sperm preparations by swim up with zeta method and density gradient centrifugation with zeta method.

Design: Experimental study.

Setting: Division of Infertility, Department of Obstetrics and Gynecology, Faculty of Medicine Siriraj hospital.

Subjects: 39 normozoospermic semen of male patients attended at the Division of Infertility, Department of Obstetrics and Gynecology, Faculty of Medicine Siriraj hospital.

Interventions: The sperm samples were randomly divided into two aliquots. One aliquot was prepared by swim up and zeta method, and the other was prepared by density gradient centrifugation and zeta method. After that, the motility rates were accessed by CASA. Then, the DNA integrity was accessed by Acridine orange staining.

Main outcome measure: The motility rate and DNA normal integrity of sperm from both groups.

Results: The sperm count decreased after preparing in both the swim up and zeta method and density gradient centrifugation and zeta method groups compared with neat semen group ($P < 0.001$). The total motility rate from swim up with zeta method was 90% and density gradient centrifugation with zeta method was 86% ($P < 0.001$). Normal sperm DNA integrity from swim up with zeta method was 95.0% and density gradient centrifugation with zeta method was 95.3% ($P = 0.582$).

Conclusion: Sperm preparation by swim-up with zeta method resulted in better sperm motility than sperm preparation by density gradient centrifugation with zeta method. The swim up with zeta method appears to be a suitable procedure to recover sperm with higher motility rate.

P019 The diagnostic usefulness of sperm DNA fragmentation tests in recurrent miscarriage: A systematic review

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Background: An association between unexplained recurrent miscarriage (RM) and sperm DNA fragmentation (SDF) has been described by various studies, but it is not yet conclusive. The predictive roles of SDF tests for spontaneous miscarriages¹ and pregnancy losses after in vitro fertilisation², have been evaluated by systematic review and meta-analysis. However, the diagnostic usefulness of SDF tests in RM has not yet been determined.

Objective: To determine the diagnostic accuracy of SDF tests in unexplained RM compared to a reference standard of retrospective clinical diagnosis of RM and proven fertility.

Methods: A systematic search was undertaken between March-April 2018; of PubMed, Cochrane, and relevant RCT registers. This was for relevant studies with the search terms 'recurrent miscarriage' AND 'sperm DNA fragmentation'. All primary diagnostic studies investigating the predictive value of SDF tests in unexplained RM, with a clearly stated reference standard, were included. There were no time limitations. Studies were assessed for methodological quality according to the QUADAS-2 checklist³ and a qualitative assessment of risk of bias and applicability was undertaken. Results of the included studies were extracted and analysed to produce likelihood ratios, the primary measure of diagnostic accuracy used in this systematic review.

Results: Three studies, comprising of 305 patients, were eligible for inclusion. Due to vast heterogeneity the diagnostic data was unable to be pooled for meta-analysis, but likelihood ratios calculated for SCD, SCSA, neutral comet and alkaline comet were 8.45 (95% CI 4.30-16.63), 7.56 (95% CI 2.01-28.43), 6.91 (95% CI 4.21-11.32) and 1.00 (95% CI 0.90-1.12), respectively. Each study was thoroughly assessed for risk of bias and applicability.

Conclusion: SDF tests, particularly SCD, SCSA and neutral comet, were measured as moderately useful in unexplained RM. However, given the described sources of bias and lack of pooled data, results should be interpreted cautiously.

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P020 Testing associations of sperm telomere length with semen parameters, clinical outcomes and lifestyle factors in human normozoospermic samples

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Background: Telomeres are repetitive DNA sequences that protect the ends of chromosomes as well as genome integrity^[1]. An increasing number of studies have found that sperm telomere length (STL) may play an important role in reproduction, sperm quality and infertility^[2-5]. Lifestyle factors can impact sperm quality and fertility^[6]. However, no studies have investigated the association of STL with lifestyle, sperm quality and Assisted Reproductive Technologies (ART) outcomes. Therefore, the aim of this study was to investigate any association between human STL with sperm parameters / clinical outcomes, and its potential link to participants' lifestyles.

Methods: Semen samples (surplus to clinical treatment) were obtained from men of couples attending a fertility clinic for ART in accordance with NHS ethics approval. They completed a detailed questionnaire about general lifestyle (food and alcohol consumption, exercise & smoking). Sperm DNA was extracted from 69 normozoospermic samples^[7] and STL was assessed using qPCR with specific primers. Pearson and Spearman correlation tests were utilised to determine associations between STL and study factors (sperm parameters/clinical outcomes/lifestyles).

Results: In the present study, STL was found positively correlated to the *in vitro* fertilisation success (n=66, r=0.359 p=0.004) but not to other clinical outcomes (embryo cleavage percentage pregnancy and live birth weight). No association was observed between STL and total sperm count, sperm concentration nor progressive motility. In our sample size, STL was not demonstrated affected by morphometric (age and Body mass index) or lifestyle factors (Smoking, Leisure score index, Healthy/Unhealthy diet score and Caffeine/alcohol consumption).

Conclusion: The results of this study demonstrate that STL is associated with *in vitro* fertilization rates, but not with semen parameters nor lifestyle factors. Further investigations are warranted to identify the potential variation of STL overtime to clarify its significance as a potential biomarker in ART.

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P021 Vaginal lubricants in the couple trying-to-conceive: Assessing healthcare professional recommendations and effect on *in vitro* sperm function

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Background: Vaginal lubricants are commonly used by couples trying-to-conceive. However, most vaginal lubricants are sperm toxic and therefore should not be used by couples trying-to-conceive. Despite this, lubricant sperm toxicity is insufficiently reported and guidance for healthcare professionals (HCPs) are absent.

Methods: In this study, lubricant-related practices of fertility-based HCPs in Scotland were sampled via an online survey. Consequently, lubricants identified as being utilised in the fertility setting were subsequently incubated with prepared sperm samples to establish effects on sperm motility.

Results: HCP recommendations (n=32) on lubricant use were varied although knowledge related to sperm toxicity was generally poor. HCPs infrequently asked about lubricant use and were unaware of guidance in this area. Aquagel, the only prescribed lubricant identified in this study, reduced sperm progressive motility to 49% of control after 10 minutes, even at concentrations as low as 5%. Vitality testing suggested the deterioration in progressive motility with Aquagel was not as a result of cell death. Conversely, Pré Vaginal Lubricant, a 'sperm-safe' lubricant, did not significantly affect any markers of sperm function assessed.

Conclusion: Vaginal lubricants should be avoided when trying-to-conceive unless specifically indicated to manage or prevent sexual dysfunction and this should be the message relayed by HCPs to patients. Development of clinical guidance in this area is recommended to ensure HCPs deliver informed advice as lubricant use in couples trying-to-conceive may inadvertently

contribute to delay in conception. Until such guidance is developed, clinics are encouraged to discuss these issues and develop a unified approach to lubricant recommendations to inform patient usage where appropriate.

P022 Assessing the impact of paternal diet on testicular morphology and apoptosis

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Background: While the association between maternal nutrition and female reproductive fitness and offspring health is well-established, the role that paternal diet plays in shaping male reproductive health is poorly understood. There is growing evidence that poor paternal diet adversely impacts sperm quality, which in turn reflects on embryonic development and offspring health. However, very few studies have investigated the effect of poor paternal diet on testicular function and morphology.

Methods: Therefore, to further characterise the effect of paternal diet on male reproductive health, we fed male mice a low protein diet (LPD) and a mineral and vitamin supplemented diet (MD-LPD).

Results: We observed that LPD-derived testes displayed increased mean total tubular area and epithelium relative to the control normal protein diet (NPD) group. On the other hand, increased tubular luminal area can be observed in response to MD-LPD. Analysis of gene expression patterns revealed that testicular expression of anti-apoptosis gene Bcl2 is increased in response to LPD and MD-LPD, whereas the expression of the pro-apoptosis gene Bax is increased in LPD-derived testes and suppressed in the MD-LPD group. Interestingly, we found that there is lesser degree of tissue apoptosis in response to paternal LPD (25.43%) compared to MD-LPD (29.47%) and NPD (39.06%).

Conclusion: This data provides further insight into testicular morphology and apoptosis in response to poor paternal diet and the possible underlying mechanisms taking place.

Donor

P023 Which donor sperm bank to choose from: Does it make a clinical difference?

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The aim of this study was to determine whether differences can be seen in fertilisation rates (FRs), IRs and CPRs in cycles where donor sperm from three different banks were used. In this retrospective study, 153 fresh IVF/ ICSI cycles using donor sperm from donor banks between August 2013 and July 2018 were analysed. Three banks were included: 30 cycles: bank 1, 22 cycles: bank 2 and 101 cycles: bank 3. FR, IR and CRP were compared. The proportion of cycles that were originally IVF but had been converted to ICSI was also compared.

Statistical analysis was performed using Fisher's Chi-square exact test and t-test ($P < 0.05$: statistically significant). Determining whether there is a difference in FRs, IRs and CPRs when using donor sperm from different banks provides clinics the opportunity to offer patients the best quality sperm available, giving them the best possible chance of success. This study revealed no significant differences in the comparable FRs between the donor banks (54.3% vs 56.7% vs 56.5%, $P > 0.05$). However, the IRs and CPRs of donor bank 2 were lower compared to the other two (IRs: 34.9% vs 17.9% vs 29.1% and CPRs: 46.7% vs 27.8% vs 48.3%), but were not statistically significant. When comparing the mean patient age from each donor bank (34.4 vs 37.4 vs 36.2) there was no significant difference. A significant difference was seen amongst the IVF/ICSI conversion rates between donor banks 1 and 3 (43.3% vs 7.9% respectively $P < 0.05$).

Data demonstrates that similar FRs are obtained using donor sperm from these 3 banks. Cycle numbers varied amongst the donor banks, therefore further data is required to confirm this finding. Due to the difference observed in IVF/ICSI conversion rates across the donor banks, it is advisable to inform patients booked in for IVF that their treatment might be converted to ICSI.

P024 The donor programme

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Background: Donor insemination treats azoospermia and helps same sex couples and single women achieve pregnancy. It is recommended that regularly ovulating women should be offered at least 6 cycles of natural cycle intrauterine insemination (NC-IUI) before considering superovulation and intrauterine insemination (SO-IUI) despite no supporting evidence^[1].

Objectives: To determine if SO-IUI confers any additional improvement to pregnancy rates compared to NC-IUI in women under 38 years and women aged 38 years or over.

Methods: 55 patients were identified from the donor programme database from August 2016 to August 2017. Cases were retrospectively reviewed using paper notes and data was collected with a standardised pro forma.

Results: 44% of women (48% of women under 38 years and 14% of women aged 38 years or over) became pregnant within 3 cycles of NC-IUI. Out of 31 women who did not become pregnant with NC-IUI, 18 had subsequent SO-IUI. 7 (39%) of these women, notably all under 38 years, became pregnant. After 3 cycles of NC-IUI followed by SO-IUI, cumulative pregnancy rates (CPR) were 56% overall, 63% for women under 38 years and 14% for women aged 38 years or over. Azoospermia (45%) was the most common indication for donor insemination. Other indications included same sex couples (40%), single women (11%) and vasectomy (4%). SO-IUI was associated with a 24% cancellation rate and 11% follicle reduction rate.

Conclusions: NC-IUI achieved a good pregnancy rate in women under 38 years and the subsequent use of SO-IUI further improved the CPR. However, this study was unable to determine whether women who conceived with SO-IUI would have conceived with additional NC-IUI. Pregnancy rates were low in women aged 38 years or over. Sample size prevents this study determining the benefits to older women of SO-IUI as opposed to NC-IUI but suggests there are none.

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P025 Engagement of healthcare providers about use of donors with expanded carrier screening during recipients' donor selection processes

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There are a variety of approaches to the genetic screening of gamete donors and donor gamete recipients. We surveyed gamete recipients to assess how they use carrier screening results and how they engage their healthcare providers in this process.

Materials and methods: An online survey tool was used to distribute the survey to clients who ordered specimens from sperm donors at our facility between October 5, 2017 and April 5, 2018 to evaluate the genetic carrier screening characteristics of donor semen recipients and their donors, and if they engaged their healthcare providers about these results.

Results: 21% of eligible clients responded to survey. Approximately one third of respondents reported that they had expanded carrier screening on themselves; 18% reported that they had carrier screening for a more limited number of genetic conditions, 42% reported that they had not had carrier screening and approximately 4% were unsure of their carrier screening. One third of clients who had any degree of carrier screening reported that they tested positive as a carrier for one or more conditions. The majority of clients reported that they did not discuss their donor's carrier screening results (64%) or the donors' family medical histories (75%) with their physicians prior to selection of their donor. This variation persisted regardless of whether the recipient or donor had ECS, and if they had positive ECS results.

Conclusions: Recipients variably engage their providers to verify their donors results are suitable for their treatment. Gamete facilities and providers may both have a role in helping to educate recipients about these types of results. Donor semen is frequently used in nations such as the UK where ECS is rarely performed. It is important to develop educational opportunities for providers about ECS in order to achieve quality patient care.

P026 Is it possible to achieve uniform gamete donor selection criteria?

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Background: Despite HFEA and BFS guidance there are variations in practice between NHS clinics in gamete donor selection criteria. Furthermore there is no system to update clinics immediately with ever changing risks of infections/diseases. This potentially exposes recipients to risk and clinics to litigation.

Objectives: To put a quality controlled process in place so that:

- A future-proofed screening questionnaire is developed to assess all gamete donors for risk of disease/infection, including travel-related infection
- Every clinic uses the same guidance, screening questionnaire and documentation to assess donors
- There are regular updates to the clinics allowing immediate action to be taken as soon as a new risk is identified.

Methods: Existing questionnaires and guidance used for screening donors were collated. A comprehensive screening questionnaire was generated for donors to fill. This questionnaire was peer reviewed by clinics and professional bodies. A pilot trial was conducted across the clinics. Feedback was obtained from donors and staff. To complement this a detailed health check guidance document to be used with the screening questionnaire was developed based on a similar format to guidelines used for blood and tissue donors. A system was established for quality control and updates of these documents.

Any new updates will be cascaded immediately to all clinics. A number of teaching sessions were held for the clinics. A champion was selected from each centre to cascade the information and train staff.

Results: A uniform donor screening questionnaire and health check guidance will be used in all NHS clinics in the region with a mechanism to quality control the documents and provide communication of any updates with immediate action.
Conclusions It is possible to establish a system of uniform donor selection criteria for gamete donors that allows a comprehensive screening.

P027 Donor egg vitrification- results from a newly created donor egg bank

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Background: Oocyte cryopreservation is now a well-established treatment modality. Improved vitrification methods have played a major role in transitioning of this technique from the experimental to the clinical stage. Vitrified donor oocytes have been shown to be at par with fresh donor oocytes^[1,2,3,4]. Supported with this knowledge base and looking at the strategies to deal with an excellent response from an egg donation campaign at the beginning of 2016, the initiative of creating a donor egg bank was taken. The aim of this study was to evaluate the effectiveness of our donor oocyte vitrification programme.

Methods: Case notes and database review of the donation cycles from which eggs were vitrified in the egg bank over a period of 2 years (April 2016- April 2018). The egg thaw survival rate, fertilization rate, number of embryos created, embryo transfer day, quality of embryo transferred, number of embryos vitrified and the outcomes of the cycles were analysed.

Results: A total of thirteen cycles were conducted with eggs from the donor egg bank. All donors were <35 years and the recipient age ranged from 27- 45 years. The number of eggs per cycle ranged from 7-17 with 5 eggs from the egg sharing cycle. The thaw survival rate was 70-100% and fertilisation rate was 60-80% following ICSI. 1-4 embryos were created from the cycles and 85% of transfers were on day three. All cycles had good grade embryos transferred and 5 cycles had additional 1-3 embryos vitrified. Positive pregnancy rate was 69% and CPR was 58%. 5 live births have been reported so far and 2 of the pregnancies are ongoing.

Conclusion: Results from our new venture on running a donor egg bank are very encouraging despite small numbers and will certainly provide us with the much needed reassurance to grow this service.

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P028 The impact of egg-sharing on live birth rates for the egg-share donor and recipient?

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Background: Egg-sharing is a scheme where a fertility patient gives a proportion of her oocytes to a recipient in exchange for free or subsidized fertility care. At the Lister Fertility Clinic, (London, UK) egg-share donors only pay the HFEA fee of £75 for their treatment, whilst the egg-share recipient pays the standard cost of fertility care plus the HFEA fee. A consistently raised concern is that the success of the egg share donor's fertility is jeopardized by donating a significant proportion of her eggs.

Aims: This study aims to compare the treatment outcome of egg-share donors and recipients with patients undergoing standard IVF treatment.

Methods: A retrospective analysis of patients participating in the egg sharing donation programme and standard IVF patients over a ten year period, between January 1st 2008 and January 1st 2017. Patients were divided into 3 groups (group 1, standard IVF/ICSI patients; group 2, egg-share donors; group 3, egg-share recipients). The standard IVF/ICSI patients were matched to the egg-share donors for age (24-35), AMH (>7) and BMI (<30).

Results: 1686 standard IVF/ICSI patients, 579 egg-share donors 724 egg-share recipients were included for analysis. Pregnancy rate was 56.2% in standard IVF/ICSI group, 58.2% in egg sharer group and 55.2% in the recipient group ($X^2=0.845$, $p=0.623$). 13.1% of the standard IVF/ICSI group, 12.4% of the egg-sharer group and 15.8% of the recipient group miscarried ($X^2=0.395$, $p=0.530$). Live birth was achieved by 43.1% of standard IVF/ICSI patients, 45.8% of egg-sharers, and 39.4% of recipients ($X^2=0.241$, $p=0.358$).

Conclusion: No significant difference in pregnancy, miscarriage, or live-birth rate was found between the groups. Therefore, patients and clinicians can be reassured that egg-sharing does not compromise pregnancy or live-birth rates for the egg sharer or the recipient when compared to standard IVF/ICSI patients.

P029 Investigating knowledge and perceptions of egg sharing among healthcare professionals in the United Kingdom

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Background: Egg sharing allows women to receive free or subsidised IVF in exchange for donating half their oocytes collected to a recipient. Although egg sharing was intended to solve the current donor oocyte shortage, egg sharing numbers have fallen over recent years in the UK. Although egg sharing is much debated among the medical community, no study has been performed formerly surveying the medical community.

Aims: To investigate the awareness and opinions of healthcare professionals' regarding egg sharing and how does this potentially effect egg sharing numbers in the UK.

Methods: Questionnaire based survey collected by convenience sampling, between September 2017 to April 2018. 304 healthcare professionals were surveyed about topics related to egg sharing, including awareness and acceptance of the programme, and relative significance of the main benefits and issues.

Results: 63.1% of respondents had little or no knowledge of egg sharing, although the majority supported the scheme once a short description was provided. Although attitudes towards egg sharing were positive, there was more support for voluntary donation. The issues surrounding egg sharing of most concern were the psychological well-being of the egg sharer if her own treatment is unsuccessful and the concern that giving away half her eggs reduces IVF success rates. Only 16.5% of respondents had previously referred a patient for egg sharing, with the majority citing lack of knowledge for the reason they hadn't referred.

Conclusion: There is currently an overwhelming lack of knowledge of egg sharing among GPs and obstetricians and gynaecologists that reduces the number of egg sharers that are informed of the programme. Education of healthcare professionals about the egg sharing programme and the research that supports it could improve their perceptions of egg sharing and egg sharing numbers.

P030 A systematic review investigating the attitudes, motivations, treatment experiences and disclosure decisions of recipients of donor oocytes

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Background: The donation of oocytes offers an answer for infertile women with certain conditions, such as primary ovarian insufficiency. In 2005 in the UK legislative changes meant that any gamete donor used to treat other people had to consent to release of their identity to any resulting offspring turning 18 years of age. Currently in the UK the supply of donor oocytes falls short of demand. There are many psychological, social and ethical issues surrounding oocyte donation, but despite this there is little research focusing on recipients of oocyte donation.

Aims: This systematic review aims to explore the motivations, attitudes, experiences of treatment and disclosure decisions from the point of view of the recipient of donor oocytes.

Methods: A systematic review following PRISMA guidelines of 3 computerized databases was undertaken. Key themes were extracted using thematic analysis.

Results: 32 studies were included for analysis. The attitudes and treatment experiences of oocyte recipients were positive, independent of treatment outcome. The majority of studies found that recipients preferred 'anonymous' egg donors to 'known' donors. The most significant characteristics of the donor requested by the recipient were race, medical history, physical appearance and intelligence. Regarding disclosure, the majority of recipients had disclosed to close family or friends of the nature of their fertility treatment. With regard to donor offspring, studies consistently reported a significant minority of recipients (approximately one third) intended not to disclose the nature of conception. Studies consistently showed a harmonious relationship between recipient and child. Recipients of different ethnic backgrounds are significantly under-represented in the literature.

Conclusion: This systematic review reports reassuring data regarding the psychological well-being of recipients during the donation process. The inconsistency of recipients informing family/friends while intending to withhold to offspring were concerning because it means multiple parties are involved in secrecy, thus risking inadvertent disclosure.

Psychosocial aspects of infertility

P031 Fertility and fatherhood in men with Cystic Fibrosis (CF)

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Purpose: CF is a life-limiting multisystem disease. Most men with CF are infertile due to congenital bilateral absence of the vas deferens. As the life-expectancy increases, decisions regarding fatherhood are becoming increasingly relevant. We set out to provide information on the current status of relationships and fatherhood in men with CF.

Methods: Single-centre cohort study of men with CF over a 10-year period.

Results: 205 men with CF attended between 2008 and 2017. Their mean age was 30.7 (16.6-64.3) years. 102 (49.5%) were single, 52 (25.7%) were married, 48 (23.3%) were in long-term heterosexual relationships, and 3 (1.45%) were in same-sex relationships. 6 men were diagnosed with CF following infertility investigations. One man had normal spermatozoa on semen analysis and fathered a child naturally. Two men had 4 adopted children and 15 men were acting as step-fathers to 20 children. 41 men and their partners underwent fertility assessment. In total 23 men had 30 children by IVF treatment following surgical sperm retrieval (n=19) or using donor sperm (n=4); 3 of these couples underwent pre-implantation genetic diagnosis (PGD). Repeated cycles of IVF were unsuccessful in 4 couples. 16 men did not proceed with treatment: in 5 of these cases their relationships ended; 2 female partners were CF carriers and are considering PGD; in some cases, there was concern about the man's prognosis; 4 couples were ineligible for NHS funding due to the female partners' children. 33 men died during the study period due to CF lung disease (n=27) and complications post-lung transplant (n=6). 3 of these men had 4 children using IVF.

Conclusion: We present comprehensive data on the current status of fertility treatments and routes to fatherhood in men with CF. Men with CF, and their partners, face complex issues and decisions when considering their relationships, fertility treatment and parenthood.

P032 Investigating patients with male factor infertility. Should we be developing appropriate, evidence based and cost-effective algorithms to guide practice?

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Background/aims: Male factor infertility is often suboptimally investigated and managed. NICE guidelines have provided recommendations for appropriate investigation of patients with male factor infertility. We reviewed the investigation and diagnosis of patients with male factor infertility and considered how we could improve this and avoid unnecessary and costly investigations.

Method: We reviewed patients attending a NHS fertility clinic over 6 months and identified patients diagnosed with male factor infertility. Patients were categorised according to sperm count as having mild (10-15m/ml), moderate (5-10m/ml), severe (1-5m/ml), and very severe (0-1m/ml) oligospermia or azospermia (0m/ml). Examinations, imaging, serum hormone levels and genetic testing were recorded.

Results: 57 patients were found to have male factor infertility. 13 patients had azospermia and 7 very severe oligospermia. 12/13 azospermic patients had FSH and testosterone levels measured while 11/13 had LH checked. 7 had elevated FSH levels and 4 had a low testosterone. Prolactin was checked in 7/13 patients and TSH in 6/13; all results were in the normal range. 8 azospermic patients had karyotype analysis with one positive result 46,XY,inv(3)(p12q23). Y chromosome microdeletions were checked in 7 patients with no positive results. 2 azospermic patients had cystic fibrosis. Of the remainder, 7/11 had PCR analysis for 50 common mutations of the CFTR gene with no positive results.

Conclusion: Patients with azospermia are not consistently investigated and may have unnecessary genetic tests costing up to £540 per patient. Investigations could be streamlined by assessing ejaculate pH and volume as well as hormone profiles prior to organising genetic testing. We are working with a consultant urologist to develop an algorithm for the investigation and diagnosis of patients with oligo and azospermia, which we will present, and which we plan to use for the education of clinicians locally and regionally.

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P033 You did not turn up... I did not realise I was invited....: understanding male attitudes towards fertility awareness and poor engagement

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Background: Involvement of men in fertility and reproductive health is important for healthy pregnancies and positive outcomes for mother, father and child. However, there is a paucity of data on men's perspective in this area. While many studies have postulated numerous reasons for lack of inclusion of men, few have actually included men. Poor engagement is

often cited as reason for this. We therefore interviewed different groups, including men, to understand the underlying reasons.

Methods: The study was a qualitative component of a wider mixed methods study. Participants were sampled from Fertility Awareness Survey respondents who agreed to follow-up interview. 35 in-depth interviews were conducted (13men, 13women and 9HCPs). Interviewees were purposively sampled to include the reproductive age-range and diverse socio-economic backgrounds. Framework analysis was utilised.

Results: We found recurring themes towards men's reluctance to engage in fertility and reproductive health discussions. The reasons different groups gave for the lack of male involvement were varied and reflected a need to evaluate different approaches for improvement. Women reported stereotypical male and female roles as barriers. They discussed the impact of societal norms and the perception that fertility is the 'woman's territory'. Healthcare professionals supported this view but also highlighted that poor male involvement was across healthcare needs and not just unique to fertility. Contrary to expectations, we found that men wanted to be involved in family building discussions and wanted to improve their knowledge. However, men felt they did not have a voice on the topic because discussions have traditionally focused on women. The notion that men were not expected to be interested and engaged thus becomes a self-fulfilling prophecy.

Conclusions: To encourage male involvement, current female-oriented services and education programmes on fertility and reproductive health should be revised to involve men. Additionally, educational programs on sexual and reproductive health should be engaging and structured to include boys and adolescents.

P034 **Affordability of assisted reproductive treatment in the public health sector of a high and low-middle income country: A mixed method study**

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Background: A number of interventions for women seeking fertility treatment have been set in place to improve their mental health, thereby increasing the pregnancy rates^[1]. However, most of these interventions fail to consider one of the most important aspects of a patient's life, prior to starting the procedure, which is their ability to fund the treatment cycle.

Methods: A cross-sectional mixed methods study surveying 116 (UK=64, Nigeria=52) IVF or ICSI-prescribed women, with a subsection of 32 (UK=15, Nigeria=17) interviewed. Affordability was assessed through survey self-reporting and during the interviews. Questionnaires were used to capture information on socio-economic status, monthly household non-food expenditure, subjective financial well-being (SFW) and out of pocket costs for the ART cycle. Payments for ART as a percentage of annual non-food expenditure was calculated to estimate catastrophic ART expenditure, factors associated with SFW were identified, and these were supplemented with a thematic analysis of the interview transcripts.

Results: In total, most of the Nigerian households incurred catastrophic expenditures, defined as an OPP of >40%^[2]; whereas, this did not occur among the UK cohort. The Nigerian households used various coping strategies which include depleting savings, borrowing money or taking contributions. Among the UK women, household income was predictive of SFW, with higher incomes associated with increased financial well-being. However, this was not observed among the Nigerian cohort. Both the UK and Nigerian women expressed similar concerns about the financial stress of funding the treatment. The key explanatory factors in both cases included that there was no certainty of a positive outcome after spending so much money, and the short-term financial constraints affected their quality of life negatively.

Conclusion: ART counselling should include financial psychoanalysis. Not all couples who are unable to afford ART in low-middle income countries, forfeit it. Cost-reducing strategies should be implemented in these settings.

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P036 **Preimplantation Genetic Diagnosis (PGD): Genuine choice for the prevention of sickle cell disease (SCD)**

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Background: SCD is a serious monogenic condition both in the UK and globally. A widely adopted approach to reduce cases involves prenatal diagnosis with a 1-2% risk of miscarriage and termination. With affected pregnancies opting against termination, PGD offers a genuine choice (with the additional possibility of affected sibling match for future transplantation).

Aim: To determine how successful PGD is for couples at risk of transmitting SCD.

Method: Couples who underwent PGD for SCD between March 2013 and October 2017 were included. Ovarian stimulation was performed using a GnRH antagonist protocol and FSH injection. GnRH agonist was used for oocyte maturational trigger. Oocytes were fertilized using ICSI. Trophoctoderm biopsy was performed on day 5 or 6 after followed by vitrification. Genetic testing was done using preimplantation genetic haplotyping.

Results: 59 at risk couples started 72 fresh PGD cycles (mean= 1.2 cycles/couple) and underwent a total of 80 frozen embryo transfers (FET) (mean= 1.3 FET/couple). 57 couples had testing for SCD only and 2 had SCD and Thalassemia major. Mean female age was 33 ± 4.4 years and AMH level was 23.5 ± 18.4 pmol/l. Mean number of oocytes retrieved was 15.8 ± 9.7 and blastocysts biopsied were 5.8 ± 4.2 . A mean of 3.4 embryos were suitable for transfer per biopsy. In 11 fresh PGD cycles, there were no embryos genetically suitable for transfer. Of the 80 FET, 82% involved a single embryo transfer resulting in 40 live births (live birth rate 50% /transfer and 68% /couple). There were three multiples (7% per live birth). There was no difference in mean female age, AMH level, number of oocytes retrieved and genetically-suitable embryos for transfer between successful (n=40) and unsuccessful (n=19) patients.

Conclusion: PGD for the prevention of SCD transmission has a high success rate and should be offered to at risk.

P037 A systematic review investigating factors determining people's intentions to undertake social egg freezing

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Introduction: Women in the UK are postponing starting a family and preventing age-related fertility decline with social egg freezing (SEF). To optimize the appropriate use of fertility preservation (FP) by egg freezing, the factors that predict women's intentions to use it should be identified and defined in the literature.

Aims: To gain an understanding of women's awareness of SEF, their intentions to use it and the reasons why women consider or undertake SEF.

Method: A systematic search of English peer-reviewed journals of three major electronic databases was undertaken. Data was analysed using thematic analysis.

Results: A total of 33 studies were included. Awareness of SEF amongst the general population is high, however specific knowledge is varied and inconsistent. The reported proportion of women who would personally consider using SEF varied widely (8.0 - 85.4%). Thematic analysis identified three key motivating factors for considering or undertaking SEF: 'lack of partner', 'insurance policy' and 'fear of future regret'. 'Career building' was also identified as a motivating factor but there was no consensus in the literature. Factors found to influence attitudes to considering SEF were 'reassurance' regarding future fertility, varying 'success rates' for eventual pregnancy and 'health risks' to future children using frozen oocytes. The authors suggest this may be due to an increased perceived risk of congenital anomalies. 'High cost' was recognized as a common concern and a perceived barrier in pursuing SEF.

Conclusions: This systematic review explores what is known regarding awareness, intentions and motivations of women considering and actively using SEF. The core themes identified provide a knowledge base to support effective counselling and informed choice for all women. Increasing the number of women pursuing SEF at an earlier stage may positively impact upon the risk of future involuntary childlessness.

P038 The fertility network UK has launched a fertility in the workplace programme encouraging all UK corporates to take on a gold standard fertility policy alongside facilitated training and workshops

Cat Strawbridge

Fertility Network UK, , UK

By introducing a Fertility Policy, which is clear, open and supportive, an employer can help the employee avoid unnecessary stress which can have a negative impact on their work, their mental health and potentially their fertility. Alongside the standard HR documents that will appear in the company handbook we want to include training and workshops to help staff and managers anticipate and manage sensitive and potentially difficult conversations so that when the time comes, they are handled successfully enabling the employee to feel confident that the employer is understanding and supportive and that the employer maintains the engagement of the employee even through this difficult time.

Examples of Fertility Policies:

- Confidentiality around fertility issues - when speaking to HR/Manager
- Flexibility around treatment - flexi hours, additional time off
- Practical solutions - allocated medication room, time allowed for time specific meds
- Understanding employees who are Childless Not By Choice
- Children in the Workplace Examples of Workshops and Training
- Talking about Fertility and Fertility Language to help create meaningful conversations

- Fertility Glossary
- Fertility Treatment Road Map to understand the process and key pressure points
- Alternative treatment options - donors, surrogates, adoption
- Workplace adjustments
- Individual Action Plans
- Independent Support Worker
- Webinars Fertility Network UK's Fertility Policy at Work programme will provide bespoke support and guidance when introducing Fertility Policies and includes initial and ongoing management and staff training opportunities which will enable your employees, at all levels, to work positively and productively when dealing with fertility issues. We encourage all fertility related organisations, as well as wider health and corporate section organisations to adopt fertility policies.

P039 A prospective qualitative study for optimisation of 'patient-centred care' in outpatient fertility services

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Purpose: The World Health Organization (WHO) recommends a 'people-centred' approach to high quality health care whereby the patient is a whole person with multidimensional needs as opposed to only managing their disease condition. The purpose of this audit was to increase our understanding of patients' experiences who attended fertility clinics between over a 4 week period in 2018 and to analyse factors leading to greater satisfaction in this group. In addition, we reviewed the problem areas to resolve these in order to improve quality of care.

Method: Patients attending the outpatient fertility clinics across four hospitals were asked to complete a paper questionnaire on the day of their appointment. This was designed to obtain the patient's perspective across five dimensions; pre-appointment indices, support services, consultation and communication, usefulness of fertility clinic introductory pack and further comments or suggestions.

Results: We had a 46% (58/126) response rate. 75% of patients found car parking was easy to find and 97% of patients felt the clinic was easy to find. Patients felt reception staff was friendlier than clinical staff. 51% of patients were seen within 30 minutes of their appointment time. Generally, patients were satisfied with the care received by their doctors across all domains. 67% of patients found the information in the fertility pack useful. Overall, 84% of responders would recommend this service to others.

Conclusions: Fertility clinics are well located and easily accessible. The majority of clinics run on time. However, clinicians should be more aware of clinic constraints and staff should better communicate delays. Overall, patients were satisfied with the care they received from doctors and would recommend the service to others.

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P040 The meaning of supportive social interactions in infertility treatment. Experimental study.

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Purpose: The aim of the research project was to analyse the importance of supporting social interactions in the process of infertility treatment. In Poland, where the study was conducted, the acceptance of ART methods is still lower than in western Europe countries, therefore the social stigma of infertility exists. The research project draws attention to the issue of disclosure of fertility problems and the ability to seek support which is a struggle for many Polish couples.

Methods: The study was conducted in an experimental model. 50 heterosexual couples qualified for IVF were recruited to take part in the study. Participants were randomly allocated between the experimental and control group. The first stage of the research procedure was carried out with participation of all couples. It included taking a saliva sample to obtain information about the level of stress based on the cortisol test. In the second stage the control group watched a non-emotional video about human embryology and the other half of participants were subject to a supportive social interaction with the participation of other couples undergoing infertility treatment. The interaction was conducted in 5 separate groups of 5 couples and was a regular conversation about couples hopes and fears. After introducing the experimental and control condition a saliva sample was again collected from all participants. Also, all participants filled psychological questionnaires regarding: perceived stress, well-being, social support and marital relations. An information about the history of infertility treatment was also collected.

Results and conclusion: The preliminary analysis indicate a statistically significantly decrease in the level of stress experienced after the supportive social interaction. The results of the analysis of differences between the experimental group and control

group as well as the relationship between the level of stress and psychological variables will be discussed during the presentation.

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P041 Plastic use in the IVF laboratory

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Plastic has been an indispensable material in the medical field. However, the true impact that plastic is having on the environment is now becoming apparent. As a result, all efforts must be made to reduce plastic waste in the medical sector. This study aimed to identify how much plastic waste is generated in the IVF laboratory at CARE London; a busy London clinic carrying out approximately 1000 fresh and 600 frozen cycles per year. The results could raise awareness within the clinic of the level of plastic used and highlight areas where plastic could be minimised.

To calculate the projected number of consumables used per year, cycle data was analysed for 3 months combined with the estimated number of each consumable used per procedure. An average was taken of these three months thus giving a prediction for the year. For packaging, the final number of consumables used was then divided by the number of consumables per packet.

The data showed that on average 69,488 plastic consumables would be used each year resulting in 22,782 pieces of packaging going to landfill sites. Furthermore, it highlighted that 14ml tubes accounted for 35% of these consumables, suggesting an area where waste could be reduced. Additionally, by altering standard operating procedures the total number of centre well dishes and 5ml round bottom tubes could be reduced by 649 and 1,354 units accounting for 14.5kg of clinical waste and 265 pieces of packaging. These changes not only benefit the environment but also decrease consumable costs. Overall, this study raised awareness of how much plastic and packaging the clinic produces per year and has resulted in processes being put into place to try make the clinic more sustainable.

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P042 New connected home ovulation test provides big data on menstrual cycles

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Background: A new home ovulation test system, Clearblue Connected Ovulation Test System (COTS) enables women seeking to conceive to monitor their fertility level via LH and estrone-3-glucuronide measurement and obtain their results on an associated mobile phone App. Users input cycle information in the App to guide testing. Users' cycle and ovulation test data is stored anonymously in the cloud, so can be analysed for big data-based insights on menstrual cycles.

Methods: Data from USA women using COTS (SPD Swiss Precision Diagnostics GmbH, Geneva) from 1st September 2017 to 21st May 2018 was analysed. This consisted of 15104 unique user IDs, 33094 cycle records and 171101 ovulation test records. Data was cleaned to remove data from validation testing, leaving 32540 cycle records. Python 3 and the relevant libraries including Pandas have been used to develop the Jupyter notebooks for this analysis.

Results: When users input their cycle length at first use, 25.3% selected a 28 day cycle, with next most common choices 27 (10.8%), 26 (10.1%) and 30 (10.0%) days. Actual cycle length was normally distributed with most common length also being 28 days, but at a lower frequency of 11.8%. Very short cycles (<23 days) were seen in 5.3% and long cycles (>44 days) in 0.9%. Of those who thought their cycle was 28 days long, 55% had their next cycle within 2 days, but 10% fell outside the range 23-44 days. 52% of users with 4 cycles data (n=534) had cycle lengths that varied by 5 or more days.

Conclusions: In one of the biggest datasets ever examined on menstrual cycles in women seeking to conceive, the cycle length distribution and variability mirrors previous studies. Some women appear to have poor knowledge of their cycles, selecting the text book length of 28 days. Those with cycles far shorter or longer than expected could be due to poor understanding of their cycle or cycle irregularity. Clearblue COTS therefore provides women with more insight on their cycles and enables them to accurately time intercourse when trying to conceive.

P043 Connected ovulation testing; user's experience

Sarah Johnson; Bola Grace; Cameron Hogg; Sharon Bench-Capon

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Background: There are only a limited number of days in a woman's cycle where unprotected intercourse can lead to pregnancy; the fertile window. Timing of intercourse to the fertile window maximises chances of natural conception, and has been shown to reduce the time to pregnancy. Home ovulation tests, especially those that measure oestrogen as well as luteinising hormone to identify the full fertile window, provide an accurate tool for timing intercourse. Apps are now very popular, but lack accuracy. A new connected ovulation test aims to combine the accuracy of hormone measurements with the convenience of an App. This study sought to examine whether a connected ovulation test, with an App, provide additional benefits to women trying to conceive.

Methods: This was a home-based study of women trying to conceive or wishing to do so in the future. Women were recruited (n=287) to use the Clearblue Connected Ovulation Test system for one menstrual cycle. This home ovulation test determined 3 phases of fertility; Low (LH and estrogen at baseline), High (estrogen rise from baseline), Peak (LH surge detected). Bluetooth connectivity enabled test results to be synced to an App. Users could add intercourse, menses and cycle data to the App and the App also indicated testing days. During the study volunteers were required to complete several questionnaires regarding usage experience; a connectivity assessment as soon as they Bluetooth pair their device (n=229), usability questionnaire after 4 weeks (n=226) and a system usability questionnaire (n=221) at study completion. Volunteers were also randomly selected for qualitative interview (n=39).

Results: Users were successfully able to connect their holders, conduct tests and sync the results to the App. Both Android and iOS users reported the App to be easy to use at the 4 week questionnaire (92.6% and 96.3% respectively). The end of study system usability questionnaire demonstrated the App to meet users requirements with 85.5% of Android users and 88.9% of iOS users scoring the App as above average. User daily usage diaries were compared to the device results stored in the Cloud and there was 99% and 100% agreement of data for Android and iOS respectively. Daily diaries also found users had a good experience, with 82.7% (Android) and 72% (iOS) of daily App experiences ranked as very positive. Most women added intercourse/menses data, with an average of 9 data entries/cycle. Qualitative interview data found some women were fearful of connecting the device at the beginning, but most found it a smooth experience. Benefits of the test were articulated as providing immediate access to data, assisting with cycle monitoring, accuracy, reminders on when to test and being able to keep data in one place.

Discussion: Women can now keep pertinent, accurate fertility information in an App, to help them conceive naturally, or if unsuccessful, share with their health care professional. The individual test data for users is available in the cloud, under data protection, so can be used to understand women's behaviour and examine cycle/fertility level characteristics in women. This can provide insights to improve tests and understand population fertility.

Nursing

P044 Positive patient experience with follitropin alfa treatment: results from a patient survey

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Background: Patient reported outcomes, such as convenience and satisfaction with treatment, are important aspects of quality of care. A patient survey investigated patient treatment experiences during use of the follitropin alfa IVF medication and injection pen.

Methods: This questionnaire-based survey included responses from 50 patients undergoing IVF treatment with follitropin alfa from May 2017. The questionnaire was completed within 15 days of commencing treatment and included 12 questions covering convenience of and satisfaction with treatment.

Results: Of the 50 patients, 48% (24/50) had experienced previous IVF treatment, whereas 52% (26/50) had not. All patients (100%; 50/50) found follitropin alfa easy to use and quick to administer and 98% (49/50) found follitropin alfa convenient to use. Overall, 98% (49/50) were satisfied with using follitropin alfa, and 78% (39/50) strongly agreed with this. The patient and product information provided with the medication was considered adequate and easy to understand by 94% (47/50) of patients. Approximately two-thirds of patients (68%; 34/50) self-administered the treatment, while the remaining patients either had their partner administer the medication for them (22%; 11/50) or the patient and their partner administered the

treatment together (10%; 5/50). Three quarters (74%; 37/50) of patients reported that travelling with their medication was an easy experience while 26% (13/50) found it neither easy nor difficult (1/13) or had no need to travel with their medication (12/13). Nearly all patients (94%; 47/50) would wish to use follitropin alfa again.

Conclusions: Patients found the follitropin alfa injection pen to be convenient and easy to use, were highly satisfied with the treatment, and nearly all would wish to use it again.

P045 A survey of specialist nursing roles in reproductive medicine

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Introduction: With rapid developments in Reproductive Medicine, increasing patient demands and limited resources, nurses are finding themselves in specialist roles, enabling more patients to have fertility treatment, and avoiding long waiting lists. Extended roles give nurses opportunities to develop new skills and enhance their career.

Aim: The aim of this survey was to look at- what roles nurses have taken on; what they would like to do in the future; what were the limiting factors and support mechanisms.

Method: All fertility nurses in our clinic filled in an anonymous questionnaire and data was collated.

Results: 14 nurses with varying experience ranging from < 1 year-34 years responded. Of these, 10 mentor new staff, 10 make clinical decisions following monitoring scans, 4 perform pelvic scans, 2 have been involved in research projects, 1 has completed the prescribing course, 1 performs embryo transfers, 1 has presented at a conference, none do egg collections or consultations and none have completed the fertility MSc (1 in progress). Of those not already doing so, nurses expressing the desire to take on specialist roles included: 10- prescribe, 9- MSc, 8-consult, 7- embryo transfers, 7-pelvic scans, 7- research projects, 4-mentor new staff, 4-egg collections, 3-make clinical decisions and none wanted to present at a conference.

Conclusion: It is encouraging to see that the nurses are taking on specialist roles and many have expressed a desire to extend their roles. Some of the limiting factors are: lack of experience/knowledge/confidence, time limitations, training opportunities and funding. In some cases the nurses thought it was not in the patient's best interest. In order to overcome these limiting factors, nurses can be given encouragement, training and support. Nurses taking on extended roles can benefit both the clinic and the patients, giving a holistic and professional service.

P046 Prediction of pregnancy outcome in relation to BHCG Levels

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Human Chorionic Gonadotrophin is produced by the body following implantation of an embryo and is used as a pregnancy indicator, either as a blood or urine test. Historically, our ladies undertook a home urine test 16 days post embryo transfer (day 2/3 embryo). However, due to increasing issues performing these tests, it was decided to perform blood tests. Initially, these were performed 14 days post transfer but led to very differing results between day 2/3 and blastocyst transfers. Currently tests are performed 17 days post egg collection for more consistency. It was hoped this would allow nursing staff to manage patient expectations with regards to pregnancy outcome.

Since June 2011, all positive tests (>5u/L) and all pregnancy outcomes including biochemical, miscarriage, ectopic, IUD, ongoing pregnancy/live birth were recorded. To date (July 2018) 1715 positive tests and outcomes have been recorded. The most recent 562 tests have been further separated into day 3 (63 tests), day 5 (499 tests) and also fresh (397 tests) and frozen (165 tests) transfers.

We found that pregnancies with a BHCG <40u/L had a 3% chance of being clinical (cardiac activity on USS), with the lowest being 37u/L (day 3 embryo). The lowest BHCG for a blastocyst transfer being 80u/L. A BHCG <150u/L had <50% chance of being clinical.

No level of BHCG predicted a miscarriage. Of the 1715 tests, 2% were ectopic with most having a starting BHCG <150u/L. The BHCG level in frozen cycles are double that of a fresh cycle to have a corresponding outcome. These results have guided our unit policy to recheck and BHCG <105u/L 48 hours later, thus allowing nursing staff to manage expectations, reduce scan appointments and predict potentially ectopic pregnancies earlier.

P047 Patient and nurse evaluation of a redesigned fertility pen injector: An international, questionnaire-based, stimulated-use study

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Objectives: To evaluate patient and nurse experiences with a redesigned, prefilled, ready-to-use multi-dose fertility pen injector (Merck KGaA, Germany).

Methods: International, simulated-use, questionnaire-based study in four European countries (UK, France, Italy, Spain) including women of reproductive age with recent or current infertility requiring assisted reproductive technology (ART) treatment (referred to as patients) and fertility nurses. Training on correct pen injector use was performed using the instructions for use accompanying the demonstration pen injector. After training, participants conducted a simulated injection into a pad and completed a questionnaire assessing their experience. Nurses received initial training and then trained an average of 2.9 patients (range 2–5). Descriptive data are summarized.

Results: 86 patients and 30 nurses were included; 65 patients had recently received/were currently receiving treatment, while 21 were naïve to ART treatment. Following training and use, 97% of patients found the pen injector easy or very easy to learn to use; no ART-treatment naïve patients rated the pen injector as difficult to use. In addition, 90% of patients agreed or strongly agreed that they would recommend the pen injector to friends and family. Regarding the nurses, all found the pen injector easy to use and teach and 90% found the overall administration process easier than expected to teach. Furthermore, 97% of the nurses would recommend the pen injector to their colleagues. 11/13 (85%) and 22/28 (79%) of nurses with previous experience with the Bemfola and Puregon pen injectors, respectively, in the 6 months before the study felt the redesigned pen injector was easier or much easier to teach. Similar results were observed when only the UK data were considered.

Conclusions: The redesigned pen injector was considered easy to use by women recently/currently receiving or requiring ART and easy to learn/teach by fertility nurses. Support: Funded by Merck KGaA, Darmstadt, Germany.

P048 How does the financial aspect of IVF treatment impact on the psychological and physical wellbeing of patients

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Purpose: In September 2017, the local Clinical Commissioning Group removed funding for IVF treatment. The reality of this means, that patients have no alternative but to self-fund their own care. IVF is a relatively high cost procedure and not affordable by all^[1]. As an NHS provider of fertility services, we developed a Nurse Led- IVF care pathway providing an effective, low cost fertility treatment.

Method: The criteria for the Nurse-Led IVF was similar for patients that would be have been suitable for NHS treatment; regular menstrual cycles, AMH between 10-50, aged 38 or under and their partner or sperm donor has a normal or slightly low sperm count. After embryo transfer, we sent a patient-satisfaction survey.

Results: From the 01/02/18 to 31/07/18; 105 enquires for the low-cost IVF were made. We treated 18 patients and 15 patients had an embryo transfer (fresh and frozen) and we achieved a pregnancy rate of 53.6 %. After embryo transfer, our survey showed that the financial aspect was rated highest for choosing Nurse - led IVF. Debt can lead to lower levels of psychological well-being^[2]: "The financial aspects of IVF treatment can be very stressful and without Nurse-Led IVF we would have either had no chance of treatment, or ended up in debt funding treatment, which would have been stressful to our marriage".

Conclusion: The Nurse-Led IVF has given our patients the opportunity to access low cost, quality IVF resulting in a 53% pregnancy rate, with no increased risk of multiple pregnancy compared with natural conception. Price-elasticity estimates suggest that a 10% decrease in IVF/ICSI cost would generate a 30% increase in utilization of IVF^[3]. We believe all IVF providers have a moral responsibility to offer patients evidenced-based and effective low-cost IVF, which results in high pregnancy rates and high patient satisfaction.

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P049 Is the role of the nurse sedation practitioner in fertility well received by patients?

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Purpose: To find out if patients had a positive experience when having an egg collection, particularly with conscious sedation administered by a nurse sedationist.

Method: 100 patients were given questionnaires covering the process from admission to discharge on the day of their egg collection. Some questions related to the actual procedure and their experience of conscious sedation.

Result: The feedback was very positive. 92% of patients rated their pain control as good or higher during the egg collection and 97% rated the experience of receiving conscious sedation as very good or excellent 2% of patients rated their pain control as poor during the procedure. These patients were noted to have increased anxiety prior to the procedure.

Conclusion: We were reassured that we were providing a good service to our patients and that the role of the nurse sedationist is effective in providing high quality and effective care for patients undergoing conscious sedation Further studies are required to compare this to patients receiving other types of sedation/anaesthesia/pain control for their egg collection <https://www.sedate-uk.com/>

Fertility preservation

P051 Protection against chemotherapy induced infertility through elevation of nicotinamide adenine di-nucleotide (NAD+)

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Maintaining female fertility during gonadotoxic chemotherapy treatment is a major challenge in the treatment of female cancer patients. The only interventions for maintaining fertility during cancer treatment rely upon the cryopreservation of oocytes, embryos or ovarian biopsies. What is needed are pharmacological options to maintain fertility. In this work, we have found in mouse studies that the metabolite nicotinamide adenine dinucleotide (NAD+) plays a critical role in protecting ovarian function from chemotherapy treatment.

Animals were treated with a single dose of doxorubicin in the presence or absence of supplementation with the NAD+ precursor nicotinamide mononucleotide (NMN), which continued for 8 weeks before functional fertility was measured by assessing ovarian reserve, oocyte yield, and breeding trials. Co-treatment with NMN successfully prevented the loss of primordial follicles, mature follicles, and maintained breeding performance during doxorubicin treatment. A similar protection against doxorubicin induced infertility was observed in a strain of transgenic animals which over-expressed the NAD+ biosynthetic enzyme NMNAT1. NMN treatment did not impair the oncological efficacy of chemotherapy, and in fact NMN alone slowed tumour growth in the MDA-MB-231 orthotopic xenograft breast cancer model more than either doxorubicin or cisplatin.

The results of this study demonstrated that ovarian toxicity induced by doxorubicin treatment can be prevented by artificially elevating NAD+ through pharmacological or genetic means. Together, this work might represent a pharmacological alternative for maintaining fertility in female cancer patients.

P053 Fertility preservation in transgender adolescents

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Background: Current guidelines encourage health professionals to address infertility risks and fertility preservation options with young people being treated for gender dysphoria and their families before starting treatment. In transitioning from male to female, GnRH-analogues ("blockers") are used to halt puberty and subsequently oestradiol is given to induce female characteristics. Side-effects include the risk of infertility or biological sterility.

Purpose: To examine fertility preservation uptake and rate of sperm banking success among transgirls seen in our Fertility Laboratory.

Methods: A retrospective audit of adolescents referred to the specialist Gender Identity Development Service (GIDS). Between 2015 and 2017, 179 transgirls attended the GIDS endocrine clinic. Fertility discussion was documented. Specialist fertility counselling was also available.

Results: 60 transgirls (34%) requested referral to our fertility laboratory. Mean age at referral was 16.4 (+/- 1.9) years. 12 transgirls were younger than 15 years (13.4 +/- 0.8). Hormonal treatment had commenced before referral in 8 cases (7 GnRH-analogue and 1 cross-sex hormones), no treatment started in 42, and no data available in 10. 51 transgirls attended the laboratory and 9 declined an appointment. A further 6 transgirls declined sperm banking following a consultation, 3 were awaiting an appointment. Of 42 transgirls attempting to bank sperm, 2(5%) were unable to provide a sample, 5(12%) were

azoospermic and 37(88%) banked sperm successfully, with a mean of 1.5 visits. Sperm parameters were variable however the majority of samples had normal concentration. The future use of sperm samples was discussed; most transgirls were unsure however 15% had considered use such as IVF/Surrogacy.

Conclusions: Our cohort of adolescent transgirls had a good rate of sperm banking success. If fertility preservation is handled sensitively in young transgirls, a high success rate can be obtained and should therefore be considered early in the transition process.

P054 Exploration of differential semen protein signatures for explaining fertility status of acute lymphoblastic leukaemia survivors

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Introduction: Acute Lymphoblastic Leukaemia (ALL) patients have a high chance of survival as a result of new treatment protocols that combines chemotherapy and radiation therapy. Infertility is one of the major consequences of these treatments. Sperm count and motility are done in routine, but they provide only a brief idea about fertility status. This study was undertaken since no proteomic based study for fertility status of treated cancer survivors was currently available which can detect or predict differential proteomics of cancer survivors.

Aims: Aim of this study was to focus on differentially expressed proteins in semen of ALL survivors with normal persons of same age group.

Material and method: The major spotlight of this study is to explore the differential proteomics of human semen to find differentially expressed proteins with normal persons of same age group. Then identification of differentially expressed proteins in ALL survivors by MALDI-TOF/MS.

Results: Total 50 samples were used in this study, out of which 30 samples were found to be having normal semen parameters. DIGE experiments were performed to spot the differentially expressed proteins in patients having normal semen parameter when compared with normal healthy and fertile controls. Out of 24 differentially expressed spots, 8 were identified by mass spectrometry. Further, three of the selected proteins were validated by western blotting and ELISA.

Conclusion: Overall, the differential expression proteomic study of semen of cancer survivors is needed to answer the role of semen proteins in maintaining the fertility of such individuals. The change in expression of these proteins in cancer survivors may provide the information about the adverse effects of cancer treatment on fertility; it will help in designing some cure related to fertility, which may further help in improving fertility in ALL survivor men. These proteins can be used in developing strategy for ALL survivors.

P055 Fertility preservation in patients with lymphoma - lessons from a tertiary clinic

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Introduction: One of the major concerns for young women when diagnosed with a condition requiring gonadotoxic therapy is for their future fertility. The challenges of treating patients with Lymphoma are their younger age, short interval from diagnosis to oncological treatment, and medical problems associated with their disease.

Aim: To assess the outcome of fertility preservation referrals for patients with lymphoma.

Material and methods: Retrospective review of referrals for fertility preservation (FP) for Lymphoma (Hodgkin's and non-Hodgkin's) between dates April 1st 2016 and August 2018. Using hospital notes and a secured electronic database we analysed progression from referral to FP treatment.

Results: From April 2016 to July 2018 71 women diagnosed with Lymphoma were reviewed. Of the cohort; 49 received a form of fertility preserving treatment; 30 had egg/embryo freezing, 16 had ovarian down regulation using GnRHagonist; 2 patients had ovarian tissue cryopreservation and 1 had ovarian transposition surgery with ovarian tissue cryopreservation. FP treatment was based on the risk of the intended oncology therapy, the urgency of treatment, and the patient's clinical condition. Of the 30 women that underwent oocyte/embryo freezing the average number of oocytes collected was 10 (0 - 21), 84% of the oocytes collected were metaphase II oocytes. The average number of embryos frozen was 5 (4 - 6) and 80% of the embryos produced were of excellent quality. The average time from consultation to oocyte collection was 17.4 days (13 - 22 days).

Conclusion: Not all patients with Lymphoma require FP treatment as the initial oncology treatment often has low a gonadotoxicity. The delay while performing FP treatment is short enough to allow intended oncology therapy to be safely deferred. Using a random start protocol we were able to obtain a good number of mature eggs.

P056 A clinician's aid to evaluating the suitability for fertility preservation

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Background: Fertility preservation is an important clinical issue with higher cancer survival rates, particularly in young adults and children. Different methodologies have been developed to help achieve this goal. Ovarian tissue cryopreservation is an emerging method of fertility preservation that is gaining momentum with recent guidance published by the British Fertility Society^[1,2]. Multiple small case series have reported positive outcomes with successful live births following orthotopic auto-transplantation.

Methods: Review of current electronic evidence databases, including review articles and guidelines, to develop a generic pathway to aid clinicians to further understand the different forms of fertility preservation currently available. We aim to present a flow chart that can serve as a reference guide for clinicians faced with this question in a clinical setting.

Results: Fertility preservation modalities currently include embryo cryopreservation, oocyte and sperm cryopreservation and ovarian tissue cryopreservation with a view to performing either orthotopic transplantation resulting in a spontaneous live birth or requiring assisted reproductive treatment with ovarian stimulation protocols to yield oocytes suitable of recovery and artificial insemination. In our model we have provided an aid for clinicians to assist patients on the best mode of fertility preservation.

Conclusions: Ovarian tissue cryopreservation is a valuable approach for fertility preservation in children, young adults and in women usually under the age of 35 undergoing gonadotoxic treatment (surgery, chemotherapy or radiotherapy). This approach has the potential to incorporate a larger cohort of patients, including those at risk of premature ovarian insufficiency (POI) (for example secondary to multiple periodic blood transfusions in transfusion dependent haemoglobinopathies) and transgender patients. The studies to date involve small case series with positive findings. In the future, cryopreserved tissue could be subjected to in-vitro maturation with subsequent in vitro fertilisation (IVF) to avoid the risk of re-transplanting malignant cells, utilising the remaining pool of primordial follicles.

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P057 Fertility preservation in women with genetic susceptibility to ovarian cancer

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Introduction: Ovarian cancer is the fifth most common cancer in females and the most fatal of all gynaecological cancers. The majority of patients are diagnosed at Stage 3 or 4. Early detection can be an effective way to reduce mortality however the lack of specific clinical symptoms in its early stage limits the sensitivity of screening methods. Women that carry germ-line mutations, BRCA1 or BRCA2 genes are at an increased risk of developing breast, ovarian and peritoneal cancer. Up to 13% of ovarian cancer patients are under the age of 45 with the majority of these genetic in origin. Offering preventive measures while preserving fertility potential can often result in a clinical dilemma. The aim of this review is to summarize the current knowledge of fertility preservation in patients at high risk of developing ovarian cancer, based on the available literature.

Method: Systematic review.

Results: Assisted reproduction techniques with embryo or oocyte cryopreservation after ovarian stimulation remains the method of fertility preservation of choice in these patients. Furthermore there is an increasing use of in vitro fertilisation (IVF) with pre-genetic diagnosis (PGD) for patients with BRCA1 and BRCA2 mutation who wish to preclude passing the mutation to the next generation. Ovarian stimulation with gonadotropins particularly in the context of IVF alone or IVF with PGD in this high risk group requires careful consideration of the background risk of developing ovarian and breast cancer. For time limited cases ovarian tissue cryopreservation followed by in vitro maturation (still experimental) or autotransplantation (live births recorded) remain controversial due to the risk of harbouring cancer cells.

Conclusion: Counselling is very important for women of childbearing age as screening is not protective while risk reducing surgery requires consideration to be given to the fertility preserving options now available.

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P059 'Predicting the unexpected' for cancer patients undergoing fertility preservation treatment

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Objective: This study assessed the predictive value of factors including ovarian reserve markers (1-3) in an attempt to identify poor and non- responders amongst oncology patients undergoing fertility preservation treatment. An accurate "Prediction model" to determine cancer patients (4) unlikely to benefit from standard fertility preservation will help minimise the risk of delaying cancer treatment.

Design: This is a retrospective cohort study examining data from 112 patients undergoing fertility preservation using random-start superovulation protocol in a tertiary IVF unit.

Materials and methods: Review of the database collected prospectively over 6 years. Statistical analysis was performed using multiple linear regression models. Analysis of variance (ANOVA) and Fisher test's significance level used was 5% ($P < 0.05$).

Results: Contributing factors were examined for poor responders (1-3 oocytes collected), normal and non-responders. These include: type and stages of cancer, starting day (of menstrual cycle) of FSH(follicle stimulating hormone), use of hormonal contraception, starting dosage of FSH; Ovarian reserve predictors: Age, AMH (Anti-Mullerian Hormone), AFC (Antral Follicle Count) and FSH. Little correlation was found between: the types and stages of cancer, the starting day of FSH, the use of hormonal contraception and the number of oocytes collected. No strong correlation was identified with age, FSH. AMH showed a strong correlation with statistical significance ($p < 0.05$) Two different AMH cut-offs were also examined (4 & 1.5), both were found to be statistically significant in predicting the poor responders. No statistical difference was found between the non-responders (no oocytes) to poor responders.

Conclusion: AMH can be used to predict who will not respond well and thus be counselled appropriately against the decision to delay their cancer treatment. No reliable predictors have been identified to predict those who will not produce any oocytes, however this study was limited by small sample size and a multicentre study is planned.

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P060 A newly introduced fertility preservation pathway: Effectiveness and outcomes

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Introduction: Oncofertility is a rapidly expanding area of fertility practise. Better cancer treatments, improved cancer survival rates and increased awareness have led to a rise in the fertility preservation referrals. A similar increase in referrals has been noted in our centre over the past few years. This led to the development of a dedicated pathway for managing these cases which included introduction of a referral form, setting up a single frequently monitored mail address for receiving the forms, identifying a team of professionals and weekly dedicated clinics/flexible appointment slots. A local seminar was organised and representative clinician from all the referring specialties were invited to inform them of this new initiative.

Objective: The objective of this study was to evaluate the effectiveness of the fertility preservation pathway since its initiation in 2015. The additional interests were to analyse the types of referral received and the outcomes of the gamete/embryo cryopreservation cycles.

Results: Data was collected for a total of 117 cases, referred from Jan 2015 to July 2017. 43 were female and 74 were male patients with predominant diagnosis of breast and testicular cancer respectively. Average of four cases were seen in a month. The pathway was effective in reducing the referral to clinic appointment time with 90% of patients seen within a week. 58% of female patients seen ($n=25$) proceeded with treatment of which 14 cycles resulted in embryo vitrification and 9 cycles resulted in oocyte vitrification. 92% of male patients ($n=62$) had successful sperm cryopreservation cycles. Informal feedback provided by the patients also highlighted a huge improvement in the satisfaction rates with the services provided.

Conclusion: Our local pathway for fertility preservation has proven to be effective in terms of reducing the waiting times for fertility preservation cases and improved patient satisfaction.

Assisted conception

P061 Does tubal patency affect intrauterine pregnancy rate following donor insemination?

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Background: Sperm donor recruitment is costly and <10% of consultations result in banked samples for treatment^[1,2]. Demand for donor sperm in the UK generally exceeds supply, with an increasing reliance on overseas sperm banks^[3,4]. Donor insemination (DI) therefore needs to be both efficacious and justifiable within an NHS setting. The impact of unilateral tubal patency on outcomes is unclear. We aimed to clarify this, to guide appropriate treatment choices for patients.

Methods: All patients undergoing DI between January 2015 and July 2018 were included. Information was collected from IDEAS electronic database. Tubal patency was assessed by hysterosalpingogram and/or laparoscopy. Treatment indication, number of cycles and treatment outcomes were recorded.

Results: 120 patients underwent a total of 436 treatment cycles. Treatment indications included same-sex couples (55%), severe male factor (28%) and single women (17%). The average age was 33 years (range 21-43 years). Cumulative pregnancy rate was 46%. Tubal patency was not assessed for 15 patients. They were therefore excluded from further analysis. 88/105 (84%) patients had bilateral tubal patency. Of these, 41 (47%) achieved a clinical pregnancy, defined as intrauterine pregnancy with fetal heart activity, following an average of 2.4 treatment cycles. Investigations did not demonstrate bilateral tubal patency in 17 (16%) patients. However, 7 (41%) achieved clinical pregnancy following an average of 2.9 treatment cycles. There was no significant difference in clinical pregnancy rate between bilateral and unilateral tubal patency ($p=0.70$). As expected, patients who did not conceive had more treatment cycles. However, there was no statistical difference between average number of cycles in those with bilateral patency (4.4; SD 1.83) compared to unilateral patency (4.1; SD 1.66) ($p=0.63$).

Conclusions: Unilateral tubal patency does not appear to affect DI success rates. This supports ongoing efforts of NHS clinics to provide DI despite challenges in sperm donor recruitment.

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P062 The outcome of endometrial perfusion with granulocyte colony-stimulating factor after two failed intracytoplasmic sperm injection cycles

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Background: Recurrent implantation failure is one of the enigmatic topics in management of subfertility using in vitro fertilization. Many therapeutic modalities have been tried to improve endometrial receptivity in such patients. Granulocyte colony-stimulating factor was triad to increase pregnancy rate in patients with recurrent implantation failure and thin endometrium.

Objective: To evaluate the effect of intrauterine infusion of G-CSF compared with placebo on pregnancy outcomes among unselected IVF/ICSI patients after at least two IVF/ICSI failed trials.

Patients and methods: This randomized controlled trial was carried out on 115 subfertile patients who had history of transfer of at least two good-quality embryos in at least two fresh or frozen IVF/ICSI failed trials. Patients were allocated randomly into two groups (59 patients in the study group 56 patients in the control group) in the study group: Patients received filgrastim (300 µg/1.0 mL, Filgrastim; SEDICO), in the control group: patients received 1.0 mL placebo (normal saline) both were administered 12 hours of hCG administration via transcervical intrauterine insemination catheter. Hysteroscopy and endometrial scratch were done for all patients in the preceding cycle. GnRH antagonist protocol in a flexible manner was used. Quantitative serum B HCG was done 14 days after embryo transfer. Transvaginal sonography was done at 5-6 weeks of gestation to document presence of intrauterine gestational sac and viable embryo.

Results: There were no significant differences between both groups in the demographic and baseline data. The biochemical pregnancy rates were (39% and 16.1%) in the study group and control group respectively (P value = 0.01). The implantation rates per 100 transferred embryos were (9.7% & 4.4%) in both groups respectively (P value = 0.03). The primary outcome of this study which was the ongoing clinical pregnancy rate was significantly higher in the study group (32.2%) than the control group (12.5%) (P value = 0.02). There was no statistically significant difference between groups, in spite of increase in

endometrial thickness before and after GCSF perfusion. There were no significant differences between the groups in the pattern of endometrium after doing logistic regression model. The strongest predictor of viable pregnancy was the pattern. A endometrium (triple line) (Relative risk=3.2).

Conclusion: Intrauterine perfusion of single-dose 300 µg GCSF 12hours before HCG trigger significantly increases the implantation, and clinical pregnancy rates in subfertile women with at least two IVF/ICSI failed trials.

P063 Diet and lifestyle factors in men undergoing microsurgical testicular extraction of sperm in a large tertiary referral centre

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Background: Identifying modifiable factors which can increase conception and live birth rates in the setting of assisted reproductive technologies (ART) is of major clinical importance. Studies investigating direct associations between diet and fertility in males undergoing fertility assessment or treatment suggest that higher intake of alcohol and red or processed meat, obesity, and smoking may lower the probability of fertilization or live birth^[1-6]. Antioxidant therapy may improve clinical pregnancy and live birth rates^[6]. Microsurgical testicular extraction of sperm (micro-TESE) is a modern technique of sperm extraction which appears to have a higher success rate in ART cases versus conventional procedures, with lower rates of complications^[7-9].

Objectives: To study the lifestyle factors of patients at our centre undergoing micro-TESE, in order to advise patients accordingly pre-procedure, and to prospectively gather data in order to compare later outcomes.

Methods: A questionnaire given to patients pre-procedure to assess the modifiable lifestyle factors suggested by the evidence base. BMI was also measured.

Results: 17 individuals have been recruited so far. 13 patients (76%) were over the 'healthy weight' range of BMI: classified as 'overweight' or 'obese'. Levels of fruit and vegetable intake were low (65% and 88% less than once per day, respectively). Sugary drink intake was high (35% at least 5-6 times a week) and exercise levels low (35% less than once a week or never). Four patients (24%) had alcohol intake levels above the NHS 'low-risk' limit. Only one was an active smoker.

Conclusions: This study provides an insight into the actual dietary habits of males undergoing micro-TESE. The results can be used to highlight potential diet and lifestyle interventions with the goal of improving fertility outcomes. Correlation with later fertility success will provide more information on the relationship between these factors in males and ART success in this relatively under-researched field.

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P064 Antioxidant supplementation, effect on sperm function and the significance of lifestyle factors: A cross-sectional study

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Background: There is a growing body of evidence surrounding the detrimental effect oxidative stress has on sperm function and male fertility. However, the evidence searching for a solution to overcome this has failed to develop at the same rate. Supplementation with oral antioxidants has been eluded to as the treatment of choice for subfertile men and an increasing number of trials are suggesting they may provide some benefit, although this evidence remains weak. Despite not being recommended by evidence at this stage, a large range of 'pro-fertility' supplements are commercially available, specifically for male fertility. It is currently unclear how many men are taking antioxidant supplementation for their fertility and the characteristics of such a group, including lifestyle factors and semen quality.

Methods: Male patients attending Ninewells Assisted Conception Unit between January 2018 to April 2018 were sampled using a short questionnaire, asking about vitamin and dietary supplement use, lifestyle factors, name, age and postcode. Sperm progressive motility and concentration were reviewed.

Results: 54 participants completed the questionnaire, showing 40% (n=22) of men were taking antioxidant supplementation, the majority for fertility purposes. Men with lower social deprivation, based on SIMD score, were more likely to be taking antioxidant supplements and were more likely to have a healthy BMI. Men who consumed less than five home-cooked meals per week were more likely to be overweight. Men with higher social deprivation consumed less home-cooked meals per week and were statistically significantly more likely to be obese.

Conclusion: Antioxidant supplements are used widely amongst male patients attending for assisted reproductive treatments. The absence of clear guidelines for clinicians and patients illustrates the need for progress in the field of male subfertility. Particularly emphasising, the need for well-designed randomised placebo-controlled, multi-centred trials, allowing stronger conclusions to be drawn regarding antioxidant supplementation.

P065 Does Total Motile Sperm Count (TMSC) affect success rates for Intrauterine Insemination (IUI) treatment?

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Introduction: The Total Motile Sperm Count (TMSC) is the number of moving sperm in the total ejaculate. Recent studies suggest that higher pre-wash TMSC is indicative of increased chance of pregnancy for patients undergoing infertility treatment. No official guidelines state what a 'normal' pre- and post-preparation TMSC is. However, using 2010 WHO 'normal' reference values for volume, concentration and motility gives a pre-preparation cut-off TMSC value of 9x10⁶, and current literature advises a TMSC of at least 5x10⁶ post-preparation for IUI treatment. The aim of this study was to assess whether a 'normal' TMSC correlated with positive pregnancy outcomes for patients undergoing IUI treatment.

Materials and methods: In a retrospective analysis, 539 stimulated IUI cycles were assessed October 2015-December 2017. The pre- and post-preparation TMSC and pregnancy outcome was recorded for each cycle. The main outcome measures were Pregnancy Rate (PR) and Clinical Pregnancy Rate (CPR). Statistical analysis was performed using Fisher's exact test (p<0.05 considered statistically significant).

Results: 89 of 539 cycles resulted in pregnancy (PR=16.5%). Of these, 21 pregnancies resulted in miscarriage, yielding a CPR of 12.6%. Above the 'normal' TMSC cut-off values there were 479 cycles, with 83 achieving a pregnancy (PR=17.3%), of which 20 resulted in miscarriage (CPR=13.2%). The remaining 60 cycles fell beneath pre- and post-preparation cut-off values (11.1% of total cycles) for what would be a 'normal' TMSC. 6 of 60 cycles resulted in pregnancy (PR=10.0%; p=0.2; NS), one of which resulted in miscarriage (CPR=8.3%; p=0.4; NS).

Conclusion: The chance of successful pregnancy in patients undergoing IUI treatment with 'sub-optimal' pre- and post-preparation TMSC values is lower than those with a 'normal' TMSC. However, the differences in CPR and PR were not statistically significant, therefore IUI treatment is still a viable option for couples with a low TMSC.

P067 Ultrasound assessment for endometrial decidualisation (the "halo sign") is a non-invasive method for assessing the effect of premature rise in serum progesterone and outcomes in IVF/ICSI

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Background: A rise in serum progesterone in the late stimulatory phase of IVF/ICSI cycles has been shown to be associated with reduced pregnancy rates¹⁻². Exposure of the endometrium to progesterone results in a characteristic appearance associated with decidualisation which is visible on ultrasound. Decidualisation progresses from the basal layer of the endometrium and should be visible on ultrasound as an echogenic layer in the endometrial myometrial junctional zone (the "halo sign"). We have assessed the association of the "halo sign" with reproductive outcomes and serum progesterone in the late stimulatory phase.

Method: This was a prospective observational study including 93 women undergoing their first IVF cycle for unexplained infertility with an antagonist protocol. All women had a single blastocyst transfer. Serum progesterone was assayed on the day ultrasound criteria were met for HCG trigger and a 3D volume of the uterus taken and then examined for the halo sign.

Results: 66 women (Group A) had no elevation in progesterone and 27 women (Group B) had a progesterone level >1.5ng/ml on the day of HCG trigger. The mean number of eggs collected, duration of stimulation and total dose of r-FSH used were not significantly different between the two groups. The pregnancy rate in women in Group A was significantly higher than Group B (44% vs 35%, P<0.05). The halo sign was seen on 18 women and the pregnancy rates in these women was significantly lower compared to those where it was absent (27% vs 42%, p<0.05). There was a significant correlation between serum progesterone and the halo sign (r=0.7, p<0.05).

Conclusion: Ultrasound visible premature decidualisation (halo sign) is a simple non-invasive method for assessing the impact of progesterone on the endometrium and correlates with pregnancy rates.

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P069 Live birth rate, ongoing pregnancy rate and ovarian hyperstimulation syndrome risk with originator versus biosimilar recombinant follitropin alfa: A pooled analysis of clinical trial data

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Objectives: To investigate whether live birth rate (LBR), ongoing pregnancy rate (OPR) and ovarian hyperstimulation syndrome (OHSS) incidence, and the benefit-risk balance of these outcomes, differ between originator and biosimilar recombinant-human follicle-stimulating hormone preparations.

Methods: Pooled analysis of phase III clinical trial data for Ovaleap (Teva BV, The Netherlands) and Bemfola (Gedeon Richter PLC, Hungary) versus GONAL-f (Merck KGaA, Germany). Primary endpoint was LBR per patient. Other endpoints were OPR per patient and OHSS incidence (any grade). Data were pooled for analysis and also evaluated separately per biosimilar to confirm trends. Endpoints were evaluated over one cycle. LBR per patient, OPR per patient and OHSS incidence were compared between originator and biosimilars (pooled) using a logistic regression model, with treatment (two categories: biosimilar or originator) and study (Ovaleap or Bemfola) included as categorical covariates.

Results: Data were included for 269 and 402 patients who received originator and biosimilars (Ovaleap, 153; Bemfola, 249), respectively. LBR was 35.7% and 43.9% with Bemfola and GONAL-f, respectively, in the Bemfola trial and 26.8% and 32.2% with Ovaleap and GONAL-f, respectively, in the Ovaleap trial. OHSS incidence was 5.6% and 3.3% with Bemfola and GONAL-f, and 4.6% and 2.7% with Ovaleap and GONAL-f. When pooled, the LBR and OPR were significantly greater with originator versus biosimilars ($p=0.0338$ and 0.0367 , respectively) and OHSS incidence was significantly lower with originator ($p=0.0109$). Differences between originator and biosimilars were independent of study (Ovaleap or Bemfola).

Conclusions: In both individual studies, LBR was at least 20% higher and OHSS incidence 40% lower with originator versus biosimilar (relative differences). Pooled analysis suggests that these differences are significant and improved outcomes may be obtained with less risk of OHSS if originator is used instead of biosimilar. Prospective clinical trials are required to support these findings. Support: Funded by Merck KGaA, Darmstadt, Germany.

P070 Hyaluronidase as a biomarker predictor of human embryo quality and pregnancy outcome

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There is increasing interest in understanding the role of the hyaluronan (HA) system in human assisted reproduction. Hyaluronidase (Hyal) cleaves high molecular weight HA into small fragments, creating ligands for RHAMM and CD44 receptors on the cell membrane and initiating cell signalling promoting cell survival and proliferation. Studies in sheep embryos showed that hyaluronidase improves the incidence and quality of blastocysts.

Objectives: To determine whether hyaluronidase is produced by human embryos and to test whether levels produced by individual embryos relate to embryo quality or pregnancy outcome.

Methods: Embryo-conditioned medium was available from couples having embryo transfer on day 2, 3 or 5. Hyaluronidase in the medium was measured by ELISA, and related to treatment outcome, stage and quality of the embryo, method of insemination, maternal age and cause of infertility. Good quality human blastocysts donated to research were immune-stained for both Hyal2 and RHAMM.

Results: ELISA showed that human embryos produce Hyal, detectable in spent culture media. Levels appeared higher in media from day 5 than day 3 embryos ($P<0.0001$) and those resulted in pregnancy ($p<0.005$). Average Hyal2 levels in media from top quality blastocysts were higher compared to poorer quality blastocysts ($p<0.005$). There was a tendency for a higher Hyal concentration in media from compacting/cavitating embryos than blastocysts ($p=0.09$). Hyal was significantly higher in IVF than ICSI blastocysts ($p<0.05$). Staining confirmed the presence of Hyal and RHAMM proteins in human blastocysts.

Discussion and conclusions: The presence and concentration of Hyal in embryo-conditioned media may have potential for prediction of pregnancy. Hyal was present in media from early stage embryos suggesting that, unlike sheep embryos, humans may produce Hyal from an earlier stage of development. Further work is required to clarify the pattern of production of Hyal and other members of HA system in human embryos at different stages.

P071 Livebirth rate is associated with oocyte yield and number of biopsied and suitable blastocysts to transfer in preimplantation genetic diagnosis (PGD) cycles: A single centre experience

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Background: It has been shown that there is a positive correlation between the number of oocyte retrieved and livebirth rate in IVF cycles^[1]. The purpose of the study was to determine whether livebirth is associated with oocyte yield and number of biopsied and suitable blastocyst to transfer following PGD to avoid transmission of monogenic disorders and unbalanced chromosomal rearrangement.

Methods: All couples referred to the IVF Center for PGD from 2014 and 2017 were included in the study. All couples had undergone controlled ovarian stimulation, blastocyst biopsy, vitrification and subsequent transfer of suitable embryo in a frozen embryo replacement (FER) cycle.

Results: 175 couples underwent PGD treatment. 249 oocytes retrievals and 230 FER cycles were carried out. The relationship between the number of oocytes retrieved and number of blastocyst biopsied was significant after adjusting for age in years ($p=0.001$; Incidence rate ratio (IRR) 1.05; 95% 1.04-1.06). Similarly, there was a significant relationship between number of oocytes retrieved and the number of suitable embryos to transfer ($p=0.001$; IRR 1.04; 95% 0.03-1.06). The total number of oocytes collected was significantly associated with the odds of a livebirth ($p=0.007$; OR 1.06; 95% CI 1.01-1.10). The total number of blastocyst biopsied and number of suitable embryos were also significantly associated with the odds of a livebirth ($p=0.001$; OR 1.14; 95% 95% CI 1.06-1.23; $p<0.001$; OR 1.38; 95% CI 1.17-1.64, respectively). There is a 14% and 38% livebirth increase per additional blastocyst biopsied and suitable embryo to transfer, respectively.

Conclusions: PGD outcome is significantly associated with oocyte yield, number of blastocysts to biopsy and suitable embryos to transfer. Fertility specialists should maximise oocyte yield in order to a maximize the number of blastocyst biopsied and suitable embryo to transfer. This will result in significantly increased odds of a livebirth following PGD treatment.

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P072 Higher oocyte numbers are not predictive of improved pregnancy outcomes

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Background: There is a long-held belief that the greater the number of oocytes retrieved, the better the pregnancy outcomes with assisted conception^[1]. This study aims to ascertain if high responders have better or worse outcomes.

Methods: A retrospective cohort study of 1483 consecutive women who had undergone a fresh IVF/ICSI cycle in a single centre was conducted.

Results: Pregnancy outcome data was collected in 1236 patients who had a day 5 embryo transferred in a fresh cycle. 334 patients were high responders (>15 oocytes collected, group A), and 670 were not (group B). There was no statistically significant difference in pregnancy rate (55% vs 51%, $p > 0.05$), live birth rate (36% versus 37%, $p > 0.05$), or miscarriage rate (12.9% versus 12.5%, $p=0.05$) between the two groups. There was a statistically significant difference in the quality of the cohort of day 3 embryos between the 2 groups, with a higher percentage of high-grade day 3 embryos in group B (80% versus 77%, $p=0.012$), but the difference was not significant for day 5 embryo quality. Antral follicle count (AFC) was age-stratified for risk of miscarriage. For the age group 26-30, there was a significant association between antral follicle count and miscarriage rate. Using binomial logistic regression, the odds of having a miscarriage increases by 1.026 with each unit increase of the AFC ($p=0.028$, 95% CI 1.003 1.050). A significant association was not noted with the other age groups tested.

Conclusions: Our data shows that women with polycystic ovarian morphology have similar pregnancy outcomes following assisted conception techniques, when compared to normal or low responders. However, our data suggests that rates of miscarriage are greater in younger women with increasing antral follicle counts, which would compromise the live birth rate in this cohort.

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P073 Live birth rate following double biopsy, vitrification and thaw of blastocysts undergoing pre-implantation genetic testing (PGT): A single centre experience

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Background: A diagnostic result is not always achieved after the first blastocyst biopsy. Subsequently, a repeat biopsy may be considered as it has been shown that a re-biopsy can yield suitable embryos to transfer^[1]. We investigate the impact of double trophectoderm biopsy, vitrification and warming on pregnancy outcomes.

Methods: All blastocyst biopsy and vitrification cycles for PGT carried out between 2014-2017 were identified. In the event couples did not have a suitable embryo to transfer, they were counselled and consented for re-warm, re-biopsy and re-vitrification. All cycles where at least one embryo yielded "no result" were included in the analysis.

Results: 682 patients underwent PGT and 4191 blastocysts were biopsied and vitrified. Of 4,191, 3,903 (93%, 95% CI 92-94) blastocysts had a result following a first biopsy and 288 (7%, 95% CI 6-8) blastocysts had "no result". 124/288 (43%, 95% CI 37-49) blastocysts were subjected to re-warm. 107/124 (86%, 95% CI 79-91) blastocysts survived the warming process. Of the 107 blastocysts which underwent double biopsy, 36 (34%, 95% CI 25-43) were suitable for transfer, 67 (63%, 95% CI 53-71) were abnormal, 4 (3%, 95% CI 1-9) had "no result". 11 couples underwent frozen embryo transfer (FET). The survival rate of embryos pre-FET was 12/13 (92%, 95% CI 67-99). The livebirth and miscarriage rate was 4/11 (36%, 95% CI 15-65) and 1/11 (9%, 95% CI 2-38), respectively. Whilst failed implantation occurred in 6/11 (55%, 95% CI 28-79) FET cycles.

Conclusions: This retrospective study has shown that it is possible to achieve live births from embryos that have undergone double warm, biopsy and vitrification. However, evidence from this study suggests that the live birth rate after a double biopsy is lower compared to embryos that have undergone a single biopsy.

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P074 Case report: Bilateral ectopic pregnancy following a double embryo transfer

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Background: Ectopic pregnancies are recognized complications of assisted reproductive technology^[1]. This case highlights a rare but important form which is reported to occur in approximately 1 per 200,000 pregnancies^[2]. Consideration of bilateral ectopic pregnancy is particularly important in cases of multiple embryo transfer. We aim to exemplify the diagnostic and therapeutic challenges this uncommon condition presents to the healthcare team.

Case description: A 33 year old presented with a 3.5 year history of primary sub fertility secondary to a significant male factor. In her third cycle of ICSI, she underwent controlled ovarian stimulation with a conventional long agonist protocol. Eight mature eggs were retrieved and following injection with donor sperm, three fertilised normally. A double embryo transfer (DET) was undertaken on day 3 of embryo development and subsequent serum HCG was positive 12 days later. At 5+1 weeks the patient presented with abdominal pain and bleeding. She was diagnosed with an ectopic pregnancy and opted for surgical management. At laparoscopy, an unruptured left sided distal ectopic was identified and salpingectomy performed. She was re-admitted 10 days later with new onset abdominal pain. Imaging revealed a haemoperitoneum and a right salpingectomy was performed for a second ruptured ectopic pregnancy.

Discussion: Delays in diagnosis of bilateral ectopic pregnancy have been discussed in the existing literature^[3]. Ultrasonography often fails to make an early diagnosis^[3,4]. Management options include bilateral salpingectomy, bilateral salpingotomy, unilateral salpingostomy with salpingectomy^[3] and systemic Methotrexate following unilateral salpingectomy^[4]. In bilateral tubal pregnancy, both fallopian tubes are likely to be irreparably damaged, therefore, Methotrexate and salpingotomy pose a risk for future tubal pregnancies and persistent trophoblast^[2].

Conclusion: Consideration of a bilateral ectopic pregnancy is of particular importance following DET. The diagnosis should be suspected in patients who present with ectopic symptoms or new pain.

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P075 Can the presence of blood or mucus on tip of embryo transfer catheter and technically difficult embryo transfer affect the IVF success rate?

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Aim: This study aims to look for the effect of blood and mucus on tip of embryo transfer catheter on the clinical pregnancy rate and live births. In addition, effect of technical difficulty of the embryo transfer and its effect on the clinical pregnancy and live births. This is a retrospective study including 4775 patients who had their IVF treatment at St. Mary's Hospital Reproductive Medicine Department over a period of 3 years from 2013 to 2016. In total of 4775 patients had Ivf cycle out of which 3610 had fresh embryo transfer. 3399 patients had easy transfer (94.1%). 211 patients (5.8%) had difficult transfers graded as per standard criteria used in unit. In patients who had easy transfer ,448 (22.3%) had blood ,123 (12.7%) had mucus and 311 (9.1%) had both mucus and blood on tip of transfer catheter. 2517 (56.8%) had no blood or mucus noted on tip of catheter. The clinical pregnancy rate was 37.5%,42.2% and 35.04% for blood, mucus and both respectively, whereas it was 37.2% with no blood or mucus noted, which showed no statistical difference. Live birth and implantation rate too had no statistical difference. In patients who had difficult transfer, clinical pregnancy rate was 24.4%,17.6% for blood & both blood and mucus respectively. In blood and mucus, clinical pregnancy rate was 29.5% without any statistical significance. Overall, the clinical pregnancy rate in patients with easy transfer was 37.18% and live birth of 33.4% whereas in difficult cases it dropped to 27.01% and 22.78% respectively.

Conclusion: Current study shows that implantation, clinical pregnancy and live birth is not affected by the presence of blood or mucus on tip of catheter. On the contrary, it is difficult technique which reduces the success of IVF rather blood or mucus on tip of catheter.

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P076 The outcome of mix grade double embryo transfer (Cleavage versus Blastocyst stage): Comparing between age group

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Background: ART conceived pregnancies have a higher risk of pregnancy loss than natural conceptions. This study was undertaken to evaluate the early miscarriage rate of double embryo transfer at blastocyst compared to cleavage stage embryos in women undergoing fresh embryo transfer, aged ≤37, and ≥38 years old.

Methods: Between June 2017 and June 2018, a retrospective cohort analysis evaluating blastocyst versus cleavage double embryo transfer in terms of miscarriage rate and multiple pregnancy rate. A total of 106 patients having undergone DET met the inclusion criteria. These were divided into 2 categories: blastocyst DET, cleavage stage DET in patients aged ≤37 and patients ≥38 years old.

Results: Patients aged ≤ 37 who received DET at the blastocyst stage had low pregnancy loss rates compared to cleavage stage (20% vs 31.2%) respectively but a higher multiple pregnancy rate; 44% as compared to 18% in the cleavage group. This was reflected with a higher biochemical pregnancy rate for the cleavage (69.5%) as compared to the day 5 DET subgroup (56%), but a similar clinical pregnancy rate (44% at blastocyst transfer versus 47% at cleavage ET p>0.05). In women ≥38 years old there was a higher biochemical and clinical pregnancy rate (67% and 40% respectively with DET blastocyst was compared to in contrast to cleavage stage DET (37% and 22.2% respectively), however, there was no statistical significant difference in terms of pregnancy loss or multiple pregnancy.

Conclusion: Our observations confirm the advantage of blastocyst transfer for all ages, and suggest that in younger women it may be associated with a lower risk of miscarriage. They also suggest that mix graded embryos' transfer does not compromise overall treatment outcome.

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P077 Hysteroscopy: Value of the method before embryo transfer in oocyte donation cycles

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Introduction: Anatomical abnormalities of the cervical and uterine cavities are associated with infertility and miscarriages and may also contribute in assisted reproduction treatment failure. Hysteroscopy is the "gold standard" method for assessing the cervical and uterine cavities.

Purpose: The aim of the above study was to assess the value of performing hysteroscopy before embryo transfer in oocyte donation treatment cycles.

Method: This is a retrospective analysis of recipients who underwent a routine diagnostic hysteroscopy prior to embryo transfer. Embryo transfer was performed at blastocyst stage of originating from fresh donor oocytes. Hysteroscopy findings and clinical pregnancy rates per embryo transfer were evaluated.

Results: Hysteroscopy findings included polyps, adhesions, small fibroids, endometritis and also anatomical abnormalities of the uterus, such as the presence of a small uterine diaphragm and cervical anomalies such as stenosis. Clinical pregnancy rate per embryo transfer in this group was 68 %. In a subgroup of patients managed with hysteroscopic polypectomy the clinical pregnancy rate per embryo transfer was 78.5 %. It is noted that X-ray Hystero-Salpingography had been reported "normal" in all cases while Transvaginal Ultrasound scanning detected the presence of polyps in only 26.3 % of cases in which there was a polyp present at Hysteroscopy.

Conclusions: This study suggests that hysteroscopy is a valuable intervention in Oocyte Donation Programmes; Hysteroscopy may detect and treat pathologies associated with implantation failure, facilitate embryo transfer through cervical dilatation in selected cases and also enhance fertility through the effect of "scratching" of the endometrium.

P078 What are the chances of a live birth following blastocyst versus cleavage stage embryo transfer in the first complete cycle of IVF?

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Background: Blastocyst transfer is increasingly favoured over cleavage stage embryos in in-vitro fertilisation (IVF)^[1]. However, as extended culture may reduce the pool of available embryos, there is uncertainty about whether this technique offers an improved chance of cumulative live-birth over a complete cycle of IVF i.e. a fresh embryo transfer followed by the replacement of any frozen embryos arising from the same episode of oocyte retrieval^[2].

Aim: To assess the chance of cumulative live-birth in the first complete cycle following a blastocyst transfer compared to cleavage stage transfer.

Methods: This population-based study used linked anonymised data from the Human Fertilisation and Embryology Authority register on IVF/ICSI treatments using autologous gametes from 1999-2011. Cumulative live-birth rates (CLBRs) were compared for couples who underwent blastocyst and cleavage transfer. Multivariable logistic regression with inverse probability of treatment weighting (IPTW) was used to estimate the effect of blastocyst versus cleavage stage embryo transfer on the odds of live-birth over the first complete cycle of IVF. IPTW allows observational studies (which are subject to treatment selection bias) to be designed similarly to randomised experiments.

Results: 100082 (91.7%) couples had a cleavage stage transfer, with 9064 (8.3%) undergoing blastocyst stage transfer. Blastocyst transfer was associated with a higher CLBR compared to cleavage stage embryo transfer (56.5% versus 34.8%). After adjusting for patient and treatment characteristics, couples who underwent blastocyst transfer had higher odds of CLBR compared to those who used cleavage stage embryos [adjusted odds ratio (95% CI): 1.58 (1.50 to 1.67)]. This advantage was maintained in single embryo transfer [AOR: 2.00 (1.78 to 2.25)], and across female age groups (<31 years=1.67 (1.50 to 1.85)); 31-35years=1.69 (1.56 to 1.83); 36-40 years=1.49 (1.35 to 1.64); >40 years=2.14 (1.40 to 3.28)).

Conclusions: Blastocyst transfer offers improved chances of live-birth in the first complete cycle of IVF/ICSI versus cleavage stage transfer.

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P079 Is using time lapse imaging incubators useful for the poor responder: A retrospective study

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Background: Poor ovarian response (defined as three or fewer oocytes obtained at oocyte retrieval) is associated with a lower prognosis following IVF and there is a lack of proven interventions to improve the outcome for these women. Time lapse imaging incubators (TLI) provide a means for better embryo assessment while minimising interruption to culture conditions and we wished to assess whether this could improve outcomes for poor responders in IVF.

Methods: We conducted a retrospective study in a single centre including all women who had a fresh embryo transfer between January 2014 and November 2017 after three or fewer oocytes being retrieved at oocyte retrieval. The primary outcome assessed was live birth rate per embryo transfer and the secondary outcomes were chemical pregnancy rate, clinical pregnancy rate, biochemical pregnancy rate and miscarriage rate. TLI used was the Embryoscope® and this was compared against IVF cycles where standard culture (SC) incubators were used. Repeat IVF cycles by the same woman in the given time period were excluded.

Results: There were 100 cycles in the TLI arm and 168 cycles in the SC arm. The live birth rate per embryo transfer was not significantly different between TLI and SC incubators (22% vs 17%, aOR 1.411, 95%CI 0.725 to 2.747). Whilst there was a trend to lower biochemical pregnancy rates (17% vs 29%, aOR 0.514, 95%CI 0.159 to 1.657) and lower miscarriage rates (12% vs 15%, aOR 0.761, 95%CI 0.155 to 3.728), this did not reach statistical significance.

Conclusions: The available data showed a trend to higher live birth rates, and lower biochemical pregnancy rates with the use of TLI for poor responders, but this did not reach statistical significance. There is a need for further research (possibly with data from multiple centres for adequate power) to provide a conclusive answer.

P080 Offspring health, growth and development following In vitro fertilisation with frozen embryos - a systematic review

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Background: Cryopreserved embryo use in in vitro fertilisation (IVF) is becoming more common because of advantages for mothers with equivalent pregnancy rates compared with fresh embryos^[1]. Evidence suggests embryo freezing is safe and may have beneficial effects for neonates^[2]. However, the effects of embryo cryopreservation on offspring health and development beyond the neonatal period are unknown and the evidence has not been reviewed.

Methods: We carried out a systematic review according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines. Eligible studies were identified from databases and reference lists, and compared health outcomes in children conceived by IVF with frozen-thawed embryos versus those conceived with fresh embryos or spontaneously. Study quality was assessed using an amended Newcastle-Ottawa scale. Between-study heterogeneity precluded a metanalysis.

Results: Of 1207 citations identified through database searches, nine studies comprising a total of 8717 participants aged up to 27 years-old from the UK, Denmark, Finland, Sweden, New Zealand, Japan and USA were included^[3-11]. Six were judged high quality and three low quality with substantial risk of bias. Five assessed cognitive development and of these, all but one low quality study found no differences between groups^[3-7]. No differences in mental health or metabolic markers of health were found^[10,11]. Two studies examined growth; one found no differences while another reported increased head circumference in girls aged one conceived from frozen embryos^[7, 11]. One study reported lower risk of benign neoplasms and eye and ear disease in the frozen embryo group compared with the fresh embryo group^[3,8,9].

Conclusions: This systematic review of nine published studies found little difference in growth, health and cognitive development between children born by frozen and fresh embryos or spontaneous conception. These reassuring findings support the safety of using frozen embryos for IVF.

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P081 Service evaluation of the efficacy and efficiency of HyCoSy for the diagnosis of tubal patency: A cost analysis study

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Background: Laparoscopy and dye test is widely accepted as the "gold standard" method for tubal patency. However, for more efficient use of resources, NICE suggests that women with no known co-morbidities should be offered Hysterosalpingography (HSG) or Hysterosalpingo-contrast-ultrasonography (HyCoSy) as a screening tool to identify who requires further laparoscopic investigation.

Objectives: The aims of this project were to evaluate the efficacy and efficiency of HyCoSy as a diagnostic tubal patency test, to calculate the cost implications of offering this procedure and the criteria for offering.

Methodology: This was a service evaluation of all women that received HyCoSy at University Hospital setting between 2014-2017. Data were analysed after being anonymised. No ethical approval was required.

Results: 200 women received HyCoSy during 2014-2017, 34 were removed because of missing or incomplete information leaving 166 women. Bilateral tubes were visualised in 83.7%, one tube was visualised in 11.4% and in 4.8% neither tube was visible. Following HyCoSy, 27% of women received Intrauterine Insemination (IUI) treatment, 43% were treated with In Vitro Fertilisation (IVF) or Intracytoplasmic Sperm Injection (ICSI) and 24% of women did not receive treatment. 1 in 20 women conceived naturally following the HyCoSy procedure.

Conclusions: HyCoSy fulfils the expectations of an infertility investigation. However, it is the decision on whether tubal patency testing is required that could be amended as a total of 92 women unnecessarily underwent HyCoSy during 2014-2017, costing £24,380 that could have potentially been avoided. As a result of this cost analysis the following local recommendations should be implemented:

- HyCoSy becomes part of the IUI treatment planning
- Reduce the age limit to 36 to be offered HyCoSy on the NHS as pregnancy rate is very low after this age with IUI
- That referral guidelines be updated and circulated in primary care.

P082 Short versus extended progesterone supplementation for luteal phase support in fresh IVF cycles: A systematic review and meta-analysis

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Background: Progesterone supplementation overcomes the luteal phase defect observed in fresh IVF cycles enabling a fresh embryo transfer. Whether it is beneficial to prolong progesterone supplementation beyond initial implantation is contentious. The objective of this study was to assess the effect of prolonged progesterone support on pregnancy outcomes in women undergoing a fresh embryo transfer after IVF/ICSI.

Methods: We included randomised controlled trials of prolonged progesterone support versus early progesterone cessation. Two independent reviewers searched EMBASE, MEDLINE and grey literature for trials from 1984 to May 2018. Risk of bias was assessed regarding randomisation, allocation sequence concealment, blinding, incomplete outcome data, selective outcome reporting, and other biases. The outcome measures were live birth rate, miscarriage rate and ongoing pregnancy rate.

Results: We included seven trials involving 1,627 participants: 3 trials reported livebirth rate with 672 live births in 830 participants, 7 trials the miscarriage rate with 178 miscarriages in 1,627 participants and 7 trials the ongoing pregnancy with 1,351 ongoing pregnancies in 1,627 participants. Clinical outcomes did not differ between patients who underwent early progesterone cessation and those who received progesterone continuation for luteal phase support in terms of live birth rate (RR: 0.94, 95% CI: 0.84-1.00), miscarriage rate (RR: 0.91, 95% CI: 0.69-1.20) or ongoing pregnancy rate (RR: 0.98, 95% CI: 0.91-1.05). Six trials were open label, which may have introduced bias and there was some heterogeneity with respect to timing of early cessation of progesterone. However, results were unchanged when we restricted analyses to those with randomization on the day of a positive hCG (RR: 0.93, 95% CI 0.83, 1.06).

Conclusions: This meta-analysis suggests that prolonged progesterone support may be unnecessary after fresh embryo transfer. Further large RCTs may be useful to corroborate this evidence and lead to a standardised duration of progesterone luteal phase support across IVF/ICSI centres.

P083 Effect of variation in laboratory and clinical practices during the last decade on IVF outcome

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Objectives: To evaluate the effect of changes in laboratory and clinical practices on IVF outcome and to estimate trend of pregnancy rates over a period of 12 years.

Methods: Review of prospectively collected data at a tertiary fertility centre. Regression analysis used to study trend in the percentage of pregnancy outcome over a period of 12 years (January 2006 to April 2018).

Results: Total number of IVF/ICSI cycles (fresh and frozen) during 12-year period (2006-2018) was 6095. Overall clinical pregnancy rate (CPR) during this period was 33.8% (n = 2065). CPRs were 36.6% (1369/3734) for fresh cycles and 35.9% (987/2749) for frozen cycles (p = 0.88). While there was no trend observed in CPR for fresh embryo transfer (ET) over 12 years, a significant increasing trend was noted in CPR with frozen ET (p < 0.05). In fresh ET cycles, a significant increase in CPR was noted when tri-gas incubators were introduced in the year 2012 (p < 0.05). In frozen ET cycles, a significant increase in CPR was noted in later years after a switch over from slow freezing to vitrification method in the year 2008 (p < 0.001). The CPRs were 18% (68/377) during slow freezing (2006-2008), 35.1% (248/706) during transition period (2009-2012) and 40.2% (671/1666) during complete switch to vitrification method (2013-2018). The overall embryo survival rate post thaw was 94% (3141/3335). Blastocyst (day 5 -7) transfer showed significantly favourable outcome when compared to cleavage stage embryo (day 2-4) ET, in both fresh and frozen cycles (p < 0.005).

Conclusion: CPR in frozen embryo transfer cycles showed an increasing trend, mainly due to successful introduction of vitrification method of cryopreservation. Use of tri-gas incubators for embryo culture and blastocyst transfer positively affected CPR.

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P084 Does ICSI provide a better outcome than conventional IVF for couples with unexplained subfertility?

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Background: While ICSI is generally recommended for moderate to severe male factor subfertility and previously reduced fertilisation rates with conventional IVF, some units use ICSI exclusively for all couples with unexplained subfertility undergoing IVF treatment.

Aim: To compare the treatment outcome following ICSI and conventional IVF performed for unexplained subfertility. Couples diagnosed with unexplained subfertility, who had split IVF and ICSI done during their treatment cycle were selected for a robust comparison.

Methods: A review of prospectively collected data on sibling oocytes collected in 137 treatment cycles inseminated by either ICSI or IVF. The embryological and clinical outcomes, in terms of fertilization rate, embryonic development, implantation rate, clinical pregnancy rate and live birth rates of sibling oocytes inseminated by IVF or ICSI in patients with unexplained infertility were evaluated. A subgroup analysis investigating the effects of split insemination on the same parameters, was on patients with different duration of unexplained infertility. Chi-square test was used to compare the groups.

Results: A total of 2332 oocytes were collected in 137 treatment cycles. 54% were inseminated by ICSI and 46% were inseminated by IVF. There was no difference of statistical significance in fertilisation rate (73.5% ICSI vs 74.2% IVF; p=0.869), embryo quality, implantation rate (41% ICSI vs 65% IVF; p=0.381), clinical pregnancy rate (41% ICSI vs 55% IVF; p=0.070) or live birth rate (30% ICSI vs 43% IVF; p=0.149). There were 5 cases of failed fertilisation in oocytes inseminated by IVF and 2 cases with ICSI insemination.

Conclusion: No clear benefit was observed by the use of ICSI over IVF as there was no difference in the embryological and clinical outcomes of insemination with IVF or ICSI in treating unexplained infertility.

P085 The impact of asynchronous syngamy on IVF outcomes

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Time lapse imaging has made it possible to investigate aspects of pronuclei (PN) syngamy. Previous studies have reported that embryos with disordered PN movement, and slow dispersion of the nuclear envelopes are less likely to result in good quality blastocysts. This study looked at the impact on syngamy synchronicity on IVF treatment outcomes.

Retrospective cohort analysis of 60 patients from one IVF Clinic in 2017. 420 embryos were cultured in an Embryoscope time lapse incubator from day 1 to day 6. Embryo videos were retrospectively reviewed to assess synchronicity of syngamy and blastocyst formation and utilisation rates. Synchronised syngamy (SS) was characterised by smooth and simultaneous disappearance of PN. Asynchronous syngamy (AS) was identified when one PN faded at least 30 mins later than the other. Blastocyst formation and utilisation rates were compared. Clinical pregnancy rates (CPR) was also noted. AS was observed in at least one embryo in 52% (26/50) of patients. The prevalence of AS in all embryos was 12% (50/420). The age of patients in for both the AS and SS groups were comparable (31.4 vs 33.4). SS rates were similar after IVF and ICSI (56% vs 44%). Day 5 blastocyst formation rates were similar in embryos showing SS and AS (67% vs 68%). Blastocyst utilisation rates, by day 6, in embryos showing SS were comparable to AS (40% vs 60%). In the AS group the CPR was 60% (3/5). This was comparable to the SS group where the CPR was 54% (26/48).

The blastocyst formation and utilisation rates for embryos showing AS are comparable to embryos demonstrating SS. While a randomised controlled trial would be required to confirm these findings and assess the impact on CPR, this study indicates that AS is unlikely to represent a key morphokinetic parameter of viability.

P086 The most useful clinical prediction models in in-vitro fertilisation: A systematic review

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Purpose/background/objectives: Prediction models in IVF may be used to inform couples of their likely chances of livebirth. However, none have found widespread use in routine clinical practice, mainly due to their limited predictive accuracy and clinical utility. We performed a systematic review of the quality and clinical utility of existing IVF prediction models to recommend the best models for clinical use.

Methods: MEDLINE and EMBASE were searched systematically for articles published from 1978 to August 2017. The review was conducted in accordance with PRISMA guidelines and the CHARMS checklist was used to extract and critically appraise the quality of included articles. We assessed correct reporting by calculating the percentage of the TRIPOD 22-checklist items met in each study.

Results: We identified 32 publications reporting on 37 prediction models. Nineteen articles had been published since the last systematic review. Five models aim were to predict the chance of pregnancy or livebirth per individual IVF cycle; the best being the Nelson and Lawlor model with the highest TRIPOD score. Two recent models (Luke and McLernon) focussed on cumulative chances of livebirth. Luke's model predicts livebirth over three fresh cycles whereas the McLernon model (which scored highest on TRIPOD) predicts livebirth over a maximum of six complete cycles (including fresh and frozen embryo transfers). The McLernon and Nelson models have been externally validated, which is essential for assessing transportability of these models to other populations.

Conclusions: This study provides a comprehensive picture of the evolving quality of IVF prediction models. Clinicians should use the most appropriate model to suit their patients' needs. We recommend the Nelson model for predicting livebirth chances at individual IVF cycles and the McLernon model for predicting cumulative livebirth over and up to six complete cycles. Both models require further external validation before use in other countries.

P087 Audit to assess if women with a diagnosis of POI who were seen in a fertility clinic setting for egg donor treatment were being provided with appropriate advice about their ongoing hormonal therapy

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Introduction: Women with Premature Ovarian Insufficiency (POI) have increased risk of osteoporosis, cardiovascular morbidity, diminished sexual wellbeing and anxiety and depression. Advice from NICE Guidelines NG23 states women should be offered hormonal treatment with Hormone Replacement Therapy (HRT) or Combined Hormonal Contraception (CHC) until at least the average age of the natural menopause (51) and given an explanation about the importance of treatment. We set out to investigate whether women with a diagnosis of POI who were seen in a fertility clinic setting for donor egg treatment were being provided with appropriate advice about their hormonal therapy and ongoing care.

Method: Standards from the NICE guidelines NG23 1.6.6 to 1.6.9 were applied regarding offering HRT or CHC until aged 51, consideration of contraindications to treatment, explanation to women about the importance of treatment. 83 sets of notes were audited from patients who had received egg donor treatment at Bath Fertility Centre between 2005 and 2016. Inclusion criteria were applied including age under 40, FSH >13, previous diagnosis of POI, history typical of menopause.

Results: 25 patients met the inclusion criteria. 0/25 (0%) met the full standards of advice as per NICE guidance 0/25 (0%) had documented evidence of contraindications to HRT 8/25 (32%) were on hormonal treatment already 7/25 (28%) had some documentation of advice about the need for HRT. Content of advice was mixed.

Conclusion: Some patients with a diagnosis of POI who had received egg donor treatment were not taking HRT or COC and did not have evidence of contraindications which would prevent use. Patients did not have documented evidence of receiving the full level of appropriate advice and information about their ongoing hormonal needs. Difficulties with the audit were noted. Advice may have been given verbally or documented at a historic appointment. They may have stopped their HRT or CHC for the short term prior to their fertility treatment.

Recommendations: This audit has highlighted the need for staff education in the fertility clinic about the importance of treatment of POI. Recommendations for service improvement include amending our treatment proforma to address ongoing hormonal needs and the need for ongoing care by a specialist with interest in menopause.

P088 Agonist Versus antagonist protocol: A review of outcomes

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Introduction: GnRH agonist and antagonist protocols have their own subset of benefits and risks. GnRH agonist protocols involve longer treatment periods, increased risk of ovarian cyst formation, menopausal symptoms and OHSS. GnRH antagonist protocols overcome many of these issues but have been associated with lower follicular production, pregnancy and implantation rates. The decision regarding which protocol to use is based on factors such as predicted ovarian reserve, risk of ovarian hyperstimulation and previous response. We carried out a review of practice to assess the impact of both stimulation protocols on fertility outcomes.

Methods: An anonymised retrospective analysis of data covering a 6-month period at a fertility unit in the South West of England was undertaken. Those 40 years and over as well as those undergoing a deferred freeze all treatment cycle were excluded from analysis.

Results: A total of 141 cycles met criteria for inclusion. Of these, 42 were antagonist cycles and 99 Agonist. The mean age of each group was 33.4 and 35.4 years respectively. The agonist group required on average 1.88 more days of stimulation than the Antagonist group ($p < 0.05$). However, there was no statistically significant difference in the proportion of top quality (TQ) embryos, Blastocyst Formation Rate or Embryo Utilisation Rate between the 2 groups. Rates of TQ embryo freezing were comparable between those undergoing antagonist and agonist protocols (45% and 43%) as were clinical pregnancy rates (52.8% Vs 53.9%).

Conclusion: Historically agonist cycles have been the preferred stimulation protocol as they were thought to be associated with better success rates. This bias in treatment preference still exists. However, these results should provide clinicians with the confidence to increasingly utilise antagonist protocols when suitable. This shift in practice would ensure a more patient focused approach with shorter treatment duration and a reduced risk of ovarian hyperstimulation.

P089 GnRH antagonist as an alternative to GnRH agonist downregulation in medicated frozen-thawed embryo transfer: Fewer clinic visits and ultrasound scans with no difference in livebirth rate

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Objectives: The optimal method of endometrial preparation for frozen-thawed embryo transfer (FET) remains uncertain^[1]. GnRH agonists are used as pituitary suppression for FET, but cause menopausal symptoms and prolong the cycle. An alternative is a GnRH antagonist. Only one study (79 cycles) has described use of GnRH antagonists in FET^[2]. The following retrospective study compares livebirth rate following GnRH antagonist and agonist FET.

Methods: Data was analysed for all medicated FETs (one per patient) between January 2014 and June 2016 during a transition between protocols. Long FET involved downregulation through administration of GnRH agonist for minimum three weeks prior to oestrogen. In short FET the antagonist was given alongside oestrogen during the first seven days. In both groups, once the endometrium reach criteria (7mm thickness) progesterone was commenced and blastocyst(s) were thawed and transferred on the sixth day. Outcomes were compared using Chi-squared or Mann-Whitney tests.

Results: 578 patients (188 antagonist, 390 agonist) were included. Cohorts were similar for BMI (mean 24.1 vs 24.0), age at egg collection (32.7 vs 32.5), age at FET (34.4 vs 34.5), embryos transferred (1.35 vs 1.38), parity (median(range) 0(5) vs 0(3)), number of oocytes in the fresh cycle (15(56) vs 15(39)) and embryo quality. Livebirth (36.7% (antagonist) vs. 39.5% (agonist), $p = 0.519$), clinical pregnancy (59.5% vs. 60.5%, $p = 0.482$) and miscarriage rates (33.3% vs. 34.3%, $p = 0.857$) were similar. In the antagonist group there were less clinic visits (median(range): 2(5) vs 3(5), $p < 0.01$) and ultrasound scans (1(3) vs 2(5), $p < 0.01$). One patient in the agonist group required cyst aspiration. Cost analysis reveals antagonist FET is 15.5% cheaper.

Conclusions: There is no difference in livebirth rate following GnRH antagonist versus agonist FET. Antagonist FET is shorter duration, cheaper and involves fewer clinic visits and scans. GnRH antagonists should be the preferred pituitary suppression in medicated FET.

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P090 Evaluation of the access AMH anti-müllerian hormone assay and antral follicle count for the prediction of ovarian response in patients undertaking their first IVF cycles

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Background: Anti-müllerian hormone (AMH) can identify patients at risk of poor ovarian response (POR) to gonadotrophin stimulation, with data from randomized controlled trials suggesting that AMH performs better than antral follicle count (AFC). The objective of this study was to evaluate the Beckman Coulter Access AMH assay for prediction of poor response, as compared to AFC and age.

Methods: A prospective cohort study of 400 patients undertaking their first IVF cycle, with data for prediction of POR (≤ 3 oocytes or cycle cancellation). AMH was measured randomly, with AFC determined on day 2-4 day of the menstrual cycle. Stimulation was performed at the discretion of the treating physician.

Results: Participants have a mean age of 33.8 ± 7.4 years with a median (IQR) AMH of 15.8 pmol/l (8.8, 28) and AFC of 12 (8, 17). The mean number of oocytes retrieved was 9.1 ± 5.2 , with 15.5% of cycles meeting the criteria for POR. The number of oocytes retrieved correlated positively with AMH (Spearman $\rho = 0.32$, $p < 0.001$) and AFC ($r = 0.39$, $p < 0.001$), and negatively with age ($r = -0.32$, $p < 0.001$). AMH and AFC were correlated ($r = 0.68$, $p < 0.001$). The median AMH among POR was 8.9 pmol/l (IQR 2.3, 21.6), with a median AFC of 8 (IQR 5, 13). For POR, AMH had an AUC of 0.64, with an optimal cutpoint of 9.9 pmol/l associated with 74% sensitivity and 55% specificity as compared to AFC with an AUC of 0.69 ($p = 0.047$ for the difference), and an AFC threshold of 9 exhibiting 70% sensitivity and 61% specificity. In the multivariable logistic regression model, AFC was the only independent predictor of POR (OR 0.87, 95%CI 0.82, 0.94).

Conclusion: The Beckman Coulter Access AMH assay was not superior to AFC in the prediction of poor ovarian response. Future studies should continue to calibrate thresholds for selection of gonadotrophin dose and clinically important outcomes.

P091 The peri-implantation endometrial fluid metabolome as a predictor of success in women undergoing in vitro fertilization treatment - a prospective cohort study

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Introduction: Metabolomics is improving the understanding of many medical conditions, but has not yet been used to study embryo implantation. The objectives of this cohort study were to investigate whether metabolomic analysis can be undertaken on endometrial fluid collected during in vitro fertilisation treatment and to identify metabolomes associated with achieving live birth.

Methods: Twenty participants were recruited. Participants underwent mock embryo transfer immediately prior to real embryo transfer. Metabolic profiling was performed on isolated endometrial fluid collected from the mock transfer catheters using direct infusion mass spectrometry analysis. Live birth rates were collected from medical records and univariate and multivariate analysis was performed to identify statistically significant fold changes in metabolites.

Results: All 20 of the endometrial fluid samples were sufficient for metabolomics analysis. A total of 33 metabolites had significant fold change between endometrial fluid collected from women achieving live birth when compared to those who did not. Importantly, metabolomic analysis can be performed on endometrial fluid, which is representative of the implantation environment.

Conclusion: A larger cohort study should be performed to further investigate endometrial fluid metabolomes that are supportive of achieving live birth in in vitro fertilisation.

P092 Comparison of clinical pregnancy rate in 2PN slow frozen versus D5/D6 vitrified blastocysts

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Aim: To compare the clinical pregnancy rates of slow frozen 2PN embryos transferred on D3-D5 and vitrified D5 or D6 blastocysts thawed and transferred on D6.

Method: The survival rates of embryos after thawing and pregnancy outcome following FET were compared retrospectively between the two cryopreservation strategies and the difference in timing of the transfer in both. Clinic data from Meditex software was retrieved retrospectively for cycle between Jan 2016- Jan 2018.

Results: A total of 332 Frozen-Thawed cycles were analysed from Jan 2016- Jan 2018. The post-thaw survival was 90% for the 2PN stage slow frozen embryos and 95% survival for the D5/D6 vitrified frozen embryos. 35 were 2PN thaws (10.5%) and 297 (89.5%) were D5/D6 thaw cycles. The Biochemical pregnancy rate for all FER cycles was 48.2% (N=160) and the Clinical pregnancy rate was 35.2% (N=117). The biochemical pregnancy rates for 2PN slow frozen and thawed was 54.2% and clinical pregnancy rate 42.8%. The biochemical pregnancy rate for D5-6 vitrified and thawed blastocysts was 47.4% and clinical pregnancy rate 34.3%. There was no statistically significant difference in pregnancy rates between the two groups.

Conclusion: The post-thaw survival rate was similar in slow freeze or Vitrification freezing methods depending on the developmental stage of embryos at freezing. There seems to be little effect on the rates of implantation and clinical pregnancy rates irrespective of the day of Embryo transfer in artificial FER cycles. Similar Biochemical and Clinical pregnancy rates with both the freezing techniques and different timing of ET was seen.

P093 Low ovarian response in young women is a quantitative not qualitative problem

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Background: Reduced ovarian reserve and oocyte yield are related to advancing maternal age. However, occasionally younger women are found to have these problems. It is unclear if a reduced ovarian reserve and subsequent egg yield is a quantitative or qualitative problem in this group. We have analysed IVF/ICSI outcomes for young women who have a reduced oocyte yield and compared this to matched older women.

Methods: This was a retrospective analysis of IVF/ICSI cycles at Hammersmith Hospital between January 2013 and December 2017 where five or fewer eggs were collected. All included patients had fresh transfers.

Results: We identified 977 cycles. Of these, 532 women were ≤ 37 years old (Group 1) and 445 were > 37 (Group 2). Women in Group 2 had a higher starting median dose of FSH [300 (IQR 300-450) iu vs 300 (IQR 200-375) iu, $p < 0.05$] and a higher total median dose of FSH used [3900 (IQR 3300-4950) iu vs 3000 (IQR 2100-4500) iu $p < 0.05$]. The median antral follicle count was higher in Group 1 [3 (IQR 1-5) vs 2 (IQR 0-3) $p < 0.05$] as was the number of eggs collected [4 (IQR 3-5) vs 4 (IQR 2-4) $p < 0.05$]. There was no difference in the fertilisation rate ($p > 0.05$), but there was a higher rate of blastocyst transfers (41% vs 21%, $p < 0.05$) in Group 1. The rate of top quality blastocyst transfers was similar in both groups (47% vs 47%). The implantation rate (40% vs 23%, $p < 0.05$) and clinical pregnancy rate (33% vs 15%, $p < 0.05$) were higher in Group 1.

Conclusion: Our data shows that ovarian reserve is a quantitative problem in young women with poor response. They should be reassured that despite the poor yield, they have a reasonable chance of success with IVF/ICSI.

P094 Unexpected poor responders have a significantly higher pregnancy rate compared to expected poor responders

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Background: Poor response to ovarian stimulation is associated with lower pregnancy rates. It is unclear whether expected poor responders have comparable outcomes to women in who a poor response is unexpected. We defined poor responders as those who had < 6 eggs collected, irrespective of the number of follicles at the last stimulation scan. We used starting dose of FSH to define anticipated ovarian response, as this is determined based on age, antral follicle count, AMH and previous response to treatment. We defined expected poor responders on the basis of a starting FSH stimulation dose of at least 300iu.

Methods: This was a retrospective analysis of consecutive IVF/ICSI cycles at a large teaching hospital between January 2013 and December 2017.

Results: We analysed 977 consecutive IVF/ICSI cycles. Of these, 702 were expected poor responders (Group 1) and 275 were unexpected (Group 2). Women in Group 1 were significantly older [median: 38 (IQR 36-39) vs 33 (IQR 31-37) years, $p < 0.05$] and a lower median antral follicle count [2 (IQR 1-3) vs 3 (IQR 1-7), $p < 0.05$]. Women in Group 2 had a higher median egg yield [4 (IQR 3-5) vs 3 (IQR 2-4), $p < 0.05$] but with no significant difference in fertilisation rates (59% vs 56%, $p > 0.05$). They were more likely to have a blastocyst transfer (52% vs 24%, $p < 0.05$) with no significant difference in the rate of top quality blastocyst transfers (50% vs 49%, $P > 0.05$). Overall, Group 2 had a significantly higher implantation rate (41% vs 28%, $p < 0.05$) and clinical pregnancy rate (33% vs 22%, $p < 0.05$).

Conclusions: Our data suggests that women with unexpected poor response have improved outcomes compared to those where a poor response is anticipated. The observed increase in pregnancy rates may be partly related to the younger age and higher egg yield. These results should be interpreted with caution as this is an observational study. Further evaluation with prospective studies is recommended.

P095 How should we manage further treatment in women who have a suboptimal response and non-conception on 300 units of FSH in IVF/ICSI cycles?

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Introduction: There is no consensus regarding the maximum dose of FSH that should be used for ovarian stimulation in IVF. The recent OPTIMIST study (van Tilborg et al, 2017) suggested no benefit when individualised high doses are administered to poor responders. Many clinicians advocate a maximum dose no greater than 300 units. There is also no agreement on how patients who respond poorly on 300 iu should be managed in future cycles.

Study design: This is a retrospective study of cycles completed between 1/1/11 and 31/12/16 in which the first cycle was conducted using 300 iu FSH and was associated with a sub-optimal response and no conception. A total of 97 patients were identified and included.

Methods: In our practice, women who have a suboptimal response and non-conception with 300 iu FSH in their first cycle of treatment are offered further treatment using 450 iu FSH. We compared the number of eggs and cycle outcome between the first cycle using 300 iu FSH in which there was a sub-optimal response and no conception and the next cycle using 450 iu FSH.

Results: Mean age at the start of the first cycle was 33.5 yrs (SD 4.1) and mean AMH 7.21 pmol/l (SD 5.00). The mean number of eggs on 300iu was 3.99 (SD 2.76). The clinical pregnancy rate, by definition, was 0% (since we only studied patients in whom there was no conception in the first cycle). The mean number of eggs on 450iu was 5.54 (SD 3.76). The difference was 1.55 eggs (95%CI 2.48 to 0.61) ($p=0.0013$). In conclusion, patients who had a suboptimal response and did not achieve pregnancy on 300iu yielded significantly more eggs on 450iu. This is statistically significant, but the clinical significance is debatable. The literature suggests a significant impact of 1-2 additional oocytes on live birth rate for poor responders (Sunkara et al, 2011). The clinical pregnancy rate in the second cycle was 10.3% and the live birth rate was 8.2%. Of the 8 patients who had their treatment cancelled due to poor response on 300 iu, 2 had a live birth in the next cycle using 450 iu. Limitations, reasons for caution: This is a retrospective observational study. The group has not been analysed regarding down regulation regime, reason of infertility or other factors that could affect ovarian response.

Conclusion: Despite the recent evidence indicating no benefit of high doses of gonadotropins for poor responders, our data suggests that there might be an additional benefit for certain patients. Further research is needed in order to quantify this benefit and balance it with the financial implications of higher FSH doses.

P096 Embryo characteristics are the most important determinants of FET outcomes

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Background: Clinical and laboratory advances in ART along with concerns about the non-physiologic peri-conception milieu of fresh IVF cycles have contributed to an increase in frozen-thawed embryo transfer (FET) cycles. Determining the factors that affect FET outcomes has become paramount to maximize ART success.

Aims/objectives: To examine FET outcomes in relation to clinical and laboratory factors.

Methods: This is a single-centre retrospective cohort study. Analysis was carried out on 246 consecutive FET cycles in 2016--2017, with embryos frozen on Day 5 or 6 of embryo development. We assessed the effect on pregnancy outcome of the following variables: female age, BMI, number of previous embryo transfers, endometrial injury in the luteal phase of the menstrual cycle prior to FET, treatment duration of oestrogen supplementation, endometrial thickness prior to progesterone supplementation, embryo development day at freezing, embryo number and grading at transfer, embryo transfer practitioner.

Results: The overall clinical pregnancy, live birth and multiple birth rates after FET cycles were 46.3%, 33.5% and 2% respectively. The embryo development day, as well as the number and grading of embryos transferred were factors that improved pregnancy outcomes after FET (Wald $\chi^2(1)=7.825$, $p=0.005$ and $\chi^2(1)=6.571$, $p=0.01$ respectively). Indeed, compared with day-5 embryos, day-6 embryos diminished the odds of positive pregnancy outcome (OR 0.172, 95%CI 0.05 to 0.591). A Kruskal-Wallis H test showed that there were differences in pregnancy outcomes between the different groups of day-5 embryos transferred, which were defined based on embryos number and grading ($H(7)=16.303$, $p=0.022$). However, after correcting for multiplicity the median FET pregnancy outcome was not statistically significantly different for either possible group combination.

Conclusions: Embryo development day as well as number and grading of embryos transferred affect independently the pregnancy outcomes in FET. Adding an embryo of lower grading did not influence FET pregnancy outcomes.

P097 Use of embryogen culture media in patients with previous natural miscarriage

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Aims/objectives: The aim of this study was to review our use of EmbryoGen culture media in patients who had no previous treatment cycles but who had previous natural conceptions which resulted in miscarriage or biochemical pregnancy.

Content of presentation: EmbryoGen is a sequential culture system enriched with GM-CSF, a cytokine associated with implantation. In a select group of patients we offer a treatment cycle supplemented with use of EmbryoGen culture media. In the majority of cases we offer EmbryoGen to patients under 40 years of age who have had at least two previously failed cycles (either failed implantation or early pregnancy loss) with good quality blastocysts replaced. However, in some cases we discuss the use of EmbryoGen on the first treatment cycle if reproductive history includes repeated miscarriage or biochemical pregnancy. During 2017 we treated 22 patients in this group who had not had any previous form of assisted conception treatment. The average female patient age was 37.6 years old, with a range from 30-42 years. All had a history of previous miscarriages, with a range of 1-6 and an average of 3.4 previous failed implantations. In this patient group eight (36%) had positive pregnancy test following treatment and five (22%) have since had a live birth. This pregnancy rate does not differ significantly from our ongoing pregnancy rate for patients on their first cycle.

Discussion: This patient group can be a difficult group to counsel as IVF treatment may not offer any obvious benefits over natural conception. This initial data set shows that the use of a GM-CSF enriched culture media can lead to successful treatment. Whether this group will benefit significantly from EmbryoGen will require further data analysis on a larger group of patients.

P098 Frozen embryo replacement cycles the risk of miscarriage is lower in a natural cycle when compared to a medicated cycle

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Background: There is a growing body of evidence to show that frozen embryo replacement cycles (FERC) show an improved pregnancy rate compared to fresh^[1]. However, there is uncertainty regarding the effect of FERC protocol on clinical outcomes. This study compared pregnancy outcome among those who had a frozen embryo replacement in a medicated cycle vs those in whom transfer was in a natural cycle.

Method: This is a retrospective cohort study of 1911 consecutive women undergoing a FERC between 2012 and 2017. Cycle and pregnancy outcome data was available and collected in 1798 patients. Results 979 patients had a positive urine pregnancy test following FERC. Of these, 876 cycles were medicated (Group A) and 103 were natural (Group B). A total of 1401 embryos were transferred in Group A and 149 in Group B, and there was no significant difference between the two groups. Whilst women in Group A had a significantly higher clinical pregnancy rate (defined as identification of a pregnancy on ultrasound scan) (n=733 vs 89, 47% vs 38%, p<0.05), there was no significant difference in live birth rates (n=490 vs 69, 31% vs 29%, p>0.05). There was no significant difference in the birth weight at delivery between the two groups (3116g vs 3157g, p>0.05). Miscarriage was defined as pregnancy loss following identification of a clinical pregnancy. The risk of miscarriage was significantly higher in Group A (n= 240 vs 17, 15% vs 7%, p<0.05). There was no significant difference in the birth weight at delivery between the two groups (3116g vs 3157g, p>0.05).

Conclusion: Our data shows that medicated FERC cycles have a significantly higher pregnancy rate but also a higher risk of miscarriage. We conclude that whilst medicated FERC protocols improve pregnancy rates, the more physiological environment in a natural cycle reduces the risk of early pregnancy loss.

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Pregnancy & outcomes

P099 HCG levels can decline pre- or post- onset of bleeding in early loss

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Objective: Non-progressive rise in hCG, followed by decline is indicative of early pregnancy loss, but it is not clear when these changes occur in relation to onset of bleeding. Tests are often conducted to check loss is complete, but rate of hCG decline is also not well characterised. This analysis examined changes in daily urinary hCG levels in relation to onset of bleeding.

Materials and methods: Home based observational cohort study (NCT01577147) following women (over 18 years old) pre-conception to early pregnancy. Volunteers collected daily urine samples for 1 entire menstrual cycle and completed daily diaries detailing menses/bleed information for around 30 days following expected period if they became pregnant. Of 348 pregnancies, 54 were reported as miscarriages at 12 week follow-up. The LH surge day was determined for miscarriages to calculate EP day and hCG measured (AutoDELFIA, Perkin Elmer, Waltham) (n=53).

Results: Almost all volunteers who reported bleeding (n=40) had hCG decline during the study period (n=38). Median day for peak hCG was 3 days after expected period (range -4 to 25) and median hCG concentration was 29.24mIU/ml (range 4-7557). Onset of bleeding occurred on average 3 days after hCG peak level, but ranged widely from 14 days before, to 10 days after hCG peak. Median time for hCG levels to decline to <1mIU/ml (baseline) from peak was 5.5 days (range 2-23 days). Time to decline was highly correlated with peak hCG concentration ($p<0.0001$). Time for hCG to decline to baseline from onset bleeding was also variable. Median was 2 days after bleeding start, but hCG levels could return to baseline as early as 25 days before onset of bleeding and up to 13 days after bleeding onset. Median days of bleeding was 5 (range 1-14), and correlated with peak hCG concentration ($p=0.0148$) and day of peak hCG concentration ($p=0.0054$).

Conclusions: Tracking of urinary hCG revealed a decline in levels prior to symptoms in many women, enabling early detection of miscarriage. Bleeding duration was also related to hCG profile. Given the heterogeneity in time of decline of hCG level, several measurements may be needed to check loss is complete. Therefore, hCG tracking could provide useful clinical information or assist setting women's expectations in early pregnancy.

P100 Evaluation of incidence, risk factors and diagnosis of miscarriage after fertility treatments: 10 years audit of clinical practice

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Objective: To assess and evaluate the incidence, diagnosis and risk factors for miscarriage after fertility treatment. Patient age, body mass index (BMI) and the number of embryos transferred back to the patient for implantation are the primary risk factors that have been evaluated, although other factors have been explored.

Methods: Retrospective audit of data collected from a database of fertility treatment cycles carried out between 2007 and 2017. All data collected was anonymised and collected per cycle rather than per patient treated.

Results: Data from 8281 fertility treatment cycles was collected and analysed. Incidence of miscarriage from the total cycles carried out was found to be 3.77% and from the total clinical pregnancies achieved the incidence was 16.08%. An overall decrease in miscarriage incidence of 1.01% was seen over the 10-year period. Patients from the age group of >40 years were shown to carry a 2.11% greater risk of miscarriage than those from the age group of <36. Cycles undertaken in patients with a lower BMI had a higher miscarriage incidence. There was an overall decrease seen in miscarriage incidence for cycles receiving both 1 and 2 embryos for implantation from 2007-2017. Cumulative data showed however, that there was a lower miscarriage incidence when only 1 embryo was transferred for implantation overall.

Conclusions: NICE guidelines suggest that 20% of all pregnancies in the general population end in miscarriage^[1]. This study shows that with assisted reproduction techniques the rate of miscarriage is lower. By analysing various subgroups of patients receiving fertility treatment we have identified those with greater risk of suffering miscarriage. This information can be used to reliably inform patients and medical professionals and aid decisions around treatment options in the future.

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P101 Ultrasound assessment of luteolysis following GnRH agonist trigger allows identification of women at increased risk of miscarriage in IVF/ICSI cycles

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Background: Luteolysis following administration of a GnRH agonist trigger has been shown to be variable between patients and it has been reported that partial luteolysis may be associated with improved pregnancy outcomes and allow better selection of patients for fresh embryo transfer ("luteal coasting").

Methods: We analysed ovarian volume 5 days after egg collection to assess degree of luteolysis and related this to reproductive outcomes in 162 consecutive women having a single embryo transfer of a top-quality blastocyst following GnRH agonist trigger. All women had intensive luteal phase support with parenteral progesterone and oral oestrogen.

Results: 82/162 (51%) women had a positive pregnancy test; of these 60/82 (73%) had an ongoing clinical pregnancy at 12 weeks gestation (Group A); the remainder (Group B) consisted of 9/82 (11%) who had a biochemical pregnancy and 13/82 (16%) who had a miscarriage. There was no statistically significant difference in the total ovarian volume (130mls vs 124mls, $p>0.05$, $t=0.466$) between women who had a positive pregnancy test and those who did not. In women who did have a positive pregnancy test, the total ovarian volume on day 5 was significantly lower (119mls vs 160mls, $p<0.05$, -1.604) in Group A compared to Group B. In these latter groups, there was no correlation between ovarian volume on day 5 and number of eggs collected ($p>0.05$) or the antral follicle count ($p>0.05$).

Conclusions: Our data shows that higher ovarian volume on day 5 is associated with an increased risk of early pregnancy loss. This increased ovarian volume did not correlate with either antral follicle count or number of eggs collected so is an apparent independent risk factor for early pregnancy loss. Further studies are required to delineate a cut off point for ovarian volume to stratify this risk.

P102 Should we treat women conceived via assisted reproductive techniques (ART) as higher risk compared to spontaneous conception? A comparison of pregnancy outcomes

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Background: The number of pregnancies conceived via IVF is increasing with HFEA quoting a 4% increase between 2015-2016^[1]. It is well known that adverse pregnancy outcomes are more common in IVF pregnancies^[2], but it is unclear how best to manage them. With this study we aim to quantify these risks and to suggest a standardised protocol for managing pregnancies conceived via ART.

Methods: We prospectively collected data on all deliveries over five years. Cohort comparisons were made between spontaneously conceived and assisted conception pregnancies. Specific outcome measures including induction of labour, method of and gestation at delivery, gestational diabetes (GDM), growth restriction (SGA), still births, shoulder dystocia, anal sphincter injury (OASIS), mean blood loss, post-partum haemorrhage (PPH) and neonatal morbidity and mortality were compared.

Results: The number of pregnancies analysed in our study were 11,875 of which 11,326 were spontaneously conceived pregnancies and 549 were conceived using ART. The mean age in the ART group was significantly higher (35.1 vs 30.1). The ART group had higher rates of adverse pregnancy outcomes such as GDM (18.9% vs 9.4%), SGA (9.1% vs 5.6%), instrumental delivery (19.6 vs 11.8%), emergency caesarean section (26.8% vs 15%), PPH >1500ml (6.9% vs 3%). There was no difference in rates of still births, shoulder dystocia and neonatal death. Higher rates of babies born with Apgar scores ≤ 6 at 5 minutes, occurred in the ART group (2.6% vs 1.4%). Admission to neonatal unit was also significantly higher in the ART group (10.2% vs 5.4%).

Conclusions: ART pregnancies do have higher pregnancy and neonatal risk factors and therefore should have further monitoring throughout the antenatal period. A standard national protocol should be considered including testing for GDM, serial growth scans, recommendation for active management of 3rd stage of labour and appropriate counselling regarding mode and timing of delivery.

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P103 Factors associated with ectopic pregnancy: Does it matter who is doing the embryo transfer?

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Background: Ectopic pregnancy (EP) has been reported to occur in 1.4-5.4% of pregnancies resulting from in vitro fertilisation (IVF). Apart from tubal disease, several factors have been shown to increase the risk of EP, such as day of embryo transfer, number of transferred embryos and fresh cycle. The aim of this study was to assess the incidence of EP and the impact of different factors that may be associated with increased rate.

Materials and methods: A consecutive series of 3702 IVF cycles resulted in pregnancy in a single tertiary assisted conception unit centre for the period between January 2015 to June 2018 were studied.

Results: During this period a total of 83 (83/3702 2.2%) pregnancies were documented as ectopic. The EP rate did not differ between D2, D3 and D5 fresh ET groups ($p>0.05$), however it was significantly higher after fresh versus frozen ET ($p<0.027$). The EP rate was found to be significantly different between eight clinicians performing ET ($P<0.05$) even when adjustment

was made for number of embryos, fresh or frozen cycle. The EP rate was not affected by type of embryo transfer catheter used. The EP rate was higher in 2016 (3.3%) in comparison with 2018 when the rate was 1.8 % ($p < 0.06$).

Conclusions: To the best of our knowledge, this is the first study to report significant difference in EP rate between clinicians performing ET. The present study demonstrates a similar EP rate following blastocyst and cleavage ETs, but is significantly reduced after frozen compared with fresh ET.

P104 Evaluation of incidence, management and risk factors of ectopic pregnancy after fertility treatments: 10 years audit of clinical practice

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Objectives and background:

- Determine the incidence of ectopic pregnancy (EP) following ART and compare with recent literature. The incidence of EP is known to rise after ART^[1].
- Assess the management of EP and compare to NICE guidelines^[2] which state that, unless certain criteria are met, EP should be managed with methotrexate.
- Assess the outcome of EP following fresh versus frozen treatment and cleavage versus blastocyst embryo transfer (ET). A recent meta-analysis^[3] found that blastocyst transfers result in a lower incidence of EP, possibly because it replicates natural conception with a shorter time to implantation^[4]. Another meta-analysis found that the incidence of EP is higher following fresh transfer, possibly because the hormones used in fresh transfer lead to overstimulation and increased contractility, affecting peristalsis thus increasing the incidence of EP^[4].

Methods: All IVF, ICSI and FET cycles over the 10 years were collated. A database search was conducted for: ectopic, tubal pregnancy, heterotopic and pregnancy of unknown location (PUL). If there was uncertainty over whether an EP had occurred, hospital records were reviewed.

Results: The EP incidence per cycle was 0.552% and incidence per positive pregnancy test was 1.82%. Most ectopic pregnancies (47.1%) were managed by salpingectomy. The EP incidence was 0.377% after FET, 0.676% after fresh ET, 0.676% after cleavage ET and 0.795% after blastocyst ET.

Conclusion: The EP incidence was slightly below the range from the literature. FET resulted in a lower EP incidence, in accordance with a recent meta-analysis. Cleavage ET resulted in a slightly lower EP incidence, disagreeing with a recent meta-analysis. Suggestions from completing the audit include checking serum hCG on day 12 post ET and repeating in 48hrs to enable early detection of EP within high risk groups and consideration of the use of FET in high EP risk groups.

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P105 Impact of assisted reproductive technology on perinatal outcomes and mode of birth: A 5-year cohort study evaluating different methodologies

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Background: Female age significantly impacts assisted reproductive treatment (ART) outcomes, with a parallel exponential increase in aneuploidy rate. There is a paucity of evidence regarding perinatal outcomes with different ART methodologies. Studies have suggested an increased preterm birth (PTB) (<37 weeks) and caesarean (CS) rate with ART, but information regarding mode of birth (MOD) with different methodologies when controlled for confounding variables (BMI, age, gestational age [GA]) is limited^[1].

Methods: Five-year retrospective cohort study: 1231 ART-related live-births; 1077 singletons; 803 and 220 occurred following IVF and ovulation induction (OI) with Clomid, respectively. Univariate analysis identified differences between ART and spontaneous births, stratified by IVF and OI. A multivariate binary logistic regression model with backward stepwise elimination identified potential confounders (age, smoking status and low-dose aspirin [LDA] at booking, GA at delivery, MOD) and determined predictors for poor obstetric outcomes (low birth weight [LBW] [<2500g]).

Results: IVF compared to OI have higher PTB rates (9.4% vs 4.8%; $p=0.035$), but comparable to the national rate (10%). IVF is an independent risk factor for PTB when adjusted for age, LDA and smoking status (OR 2.08,95%CI1.03-4.21). Clomid compared to IVF: 25% (OR1.25,95%CI1.12-1.39; $p<0.01$) greater GA; lower median birth weight after adjustment for GA (3378g vs 3430g; OR0.999,95%CI0.999-1.00; $p<0.01$) and lower LBW rate (5.2% vs 6.4%; $p=0.63$) comparable to national rate (5%); higher incidence of low APGAR's (<7 at 5 minutes of birth) (2.4% vs 0.6%; $p=0.039$); and, higher CS rate (28.9% vs 27.7%; $p=0.021$), however, significance was not maintained when corrected for GA (OR0.71,95%CI 0.51-1.00; $p=0.05$). Age and BMI at booking are independent risk factors for CS (OR1.10,95%CI 1.065-1.12 and OR1.07, 95%CI 1.037-1.10, respectively) when adjusted for GA.

Conclusions: ART does not adversely affect pregnancy outcomes when adjusted for confounders. It has a significant impact on service delivery provision with ART.

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P106 Should ART clinics move towards a freeze all embryo policy for every patient?

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Introduction: An increasingly controversial subject in ART is whether IVF clinics should adopt a freeze all embryo (FAE) approach to improve success rates. FAE strategy is driven by a multitude of concerns including compromised endometrial receptivity during fresh cycles. However, previous studies have reported contradictory outcomes to FAE approach, and may not always be applicable to individual clinical contexts.

Method/results: This retrospective study analysed all 936 fresh and 359 frozen cycles performed between January 2017 and July 2018 of which 630 and 256 cycles were eligible for analysis. Preliminary findings demonstrated superior implantation rates (IR) in frozen cycles across all age groups with significant improvement in patient's aged 35-39 ($P=0.001$). However, further investigation revealed patient cohort bias as fresh cycles included transfers on day 3/5 with reduced embryo quality whereas in frozen cycles, only good quality blastocysts were transferred. When comparing fresh cycles in which embryos were frozen ($n=69$) vs cycles in which none were ($n=100$), cycles with freezing concluded more frequently in blastocyst transfer (95%vs51%), had fewer embryos replaced (1.2vs1.5) and higher CPR (44.9%vs35.0%). Addressing this bias, the data were reanalysed comparing all eSET fresh and frozen cycles ($n=242$ vs146). No significant differences in IR and CPR in any age group were noted.

Conclusion: Data analysis appears to demonstrate significantly improved outcomes following FAE, but may reflect significant biases. When addressed, these success rates appeared similar. This is consistent with findings of multiple recent large RCTs and does not support implementing FAE as routine practice, if the sole aim is to increase pregnancy rates. Although this is a retrospective study that may be open to confounding factors, it does provide information pertinent to clinical decision-making in our centre. Further research is required to identify into specific groups who may benefit such as patients with over 15 oocytes or elevated progesterone levels.

P107 Pregnancy outcome in fresh and frozen embryo transfers following GnRH trigger

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Background: In IVF treatment, GnRH trigger is used to reduce the risk of Ovarian Hyper Stimulation Syndrome (OHSS) in high responders. Most fertility units have their own protocol to decide whether to go ahead with fresh embryo transfer (ET) with additional luteal support or electively freeze all the embryos. One recommendation to avoid the risk of OHSS with these women is to electively freeze the embryos then do frozen ET. With advances in freezing/thawing techniques, the pregnancy outcome is better with frozen ET. It is also not clear whether the additional luteal support given in fresh ET is efficient enough.

Objectives: To assess the pregnancy outcome in fresh and frozen ET following busarelin (GnRH agonist) trigger. To assess the OHSS admissions following fresh ET with busarelin trigger.

Methods: Retrospective analysis of women who had busarelin trigger (0.5mg) from November 2015 - 16 were analysed.

Results: 133 women were included in the study. 52/133(39%) women had fresh ET and 81/133(61%) had frozen ET. The women who proceeded with fresh ET were given 1500 iu of HCG on the day of egg collection and cyclogest pessaries twice daily from the day after the egg collection as additional luteal support. In Fresh ET group, positive pregnancy test rate was 65% (34/52), pregnancy loss was 23% (12/52) and clinical pregnancy was 42% (22/52). 2 patients had OHSS admission in fresh ET group. In Frozen ET group, positive pregnancy test rate was 62% (50/81), pregnancy loss was 17% (14/81) and clinical pregnancy was 44% (36/81). No patients had OHSS admission in frozen ET group.

Conclusion:

- Better clinical pregnancy rate (44% vs 42%) and lower pregnancy loss (17% vs 23%) were noted in frozen ET
- No women needed admission for OHSS with frozen ET
- Elective freeze all should be considered.

P108 Frozen embryo replacement cycles versus fresh cycles; a comparison of perinatal outcomes

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Previous studies suggest that Assisted Reproduction techniques could have an impact on perinatal outcomes. There is conflicting data on the effect of embryo cryopreservation on birth and perinatal outcomes. The aim of this study was to compare the effects of fresh embryo transfer (ET) versus frozen ET (FET) on clinical pregnancy rate (CPR), gender ratio, mode of delivery, gestational age birthweight and neonatal deaths.

We performed a retrospective analysis of fresh and frozen cycles at Hammersmith Hospital between October 2010 and November 2017. Day 2 and Day 3 transfers as well as treatments with slow-frozen embryos were excluded. 3,622 ETs and 1,711 FETs were compared for CPR. For perinatal outcomes, non-singleton pregnancies were excluded. Perinatal outcomes for 1,061 and 413 single live births after fresh ETs and FETs were analysed.

All deliveries were grouped into two groups: vaginal normal delivery and caesarean section. Low birthweight was considered $\leq 2500\text{kg}$ and preterm labour $< 37\text{weeks}$ as defined by World Health Organization (WHO) and American College of Obstetricians and Gynaecologists (ACOG) guidelines. Fisher's Chi-square exact test and t-test were used for statistical significance. $P < 0.05$ was considered statistically significant. CPR for fresh ETs was significant higher compared to FETs, (43.62% vs 38.51%, $p = 0.0005$). Also, a significant higher amount of normal vaginal deliveries was observed after fresh ETs (62.58% vs 53.51%, $p = 0.0017$). Fresh ETs were associated with significant lower mean birthweight compared to FETs (3175.33kg vs 3334.55kg, $p = 0.0005$). However, non-significant differences were observed between fresh ETs and FETs for gender ratios (49.19% females vs 48.66%, $p = 0.8620$), gestational age (11.21% preterm deliveries vs 11.38%, $p = 0.9271$), neonatal deaths (0.37% vs 0.48%, $p = 0.6747$) and still births (0.09% vs 0.72%, $p = 0.0691$).

Our study showed that laboratory techniques such as embryo cryopreservation do have an impact on perinatal outcomes which requires further investigation as our study was a retrospective analysis.

P109 Fertility outcome of women undergoing hysteroscopic fibroid resection is not dependent on resection of single or multiple fibroids

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Resected, cavity-distorting submucous fibroids increase clinical pregnancy rates in women undergoing fertility treatment. However, there is insufficient evidence on extent of surgery and impact on live birth or miscarriage rate. This study's question is whether fertility outcome is better in hysteroscopic resection of single fibroids compared to multiple fibroids.

Methods: This is a 7-year cohort study of women, who had a hysteroscopic resection for fibroids through our Centre of Reproductive Medicine (2011-2017). In this period, 31 women underwent this procedure as part of their fertility treatment. Data was collected using our online patient health-record system and medical notes.

Two groups were defined; Group 1 ($n = 20$) consisted of women who had a single fibroid resected and Group 2 ($n = 11$) included patients who had multiple fibroids resected. The outcomes were defined as pregnancy beyond 12 weeks, failed IVF/ICSI treatment and not pregnant. Data was analysed using the Chi-Square test.

Results: There was no difference in the demographics of both groups. Mean age is 37 years (Group 1) and 38 years (Group 2) and average duration of subfertility was 3 years (Group 1) and 2.8 years (Group 2). In this cohort, there was no significant difference in fertility outcome between Group 1 and 2 ($p = 0.11$). We also compared the classification of fibroid, defined as type 0 (complete submucous), type 1 (50% submucous) and type 2 (less than 50% submucous), to fertility outcome. There was no significant difference between the types of fibroids resected in fertility outcome ($p = 0.44$).

Conclusions: Surface area of resection due to multiple fibroid resection, does not appear to influence pregnancy outcome. Limitations of this study is the small sample size, despite study period of 7 years. The authors recommend a combined effort of multiple UK centres to collate data on these procedures to provide meaningful answer to this study's question.

P110 Maternal protein restricted diet alters fetal immune system

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Purpose/background/objectives: In the Australian cattle industry, bacterial and parasite infections and viral diseases represent a huge economical loss^[1]. It has been demonstrated that maternal malnutrition has an impact on the development of offspring immunity^[2-4]. Since the thymus is a pivotal organ within the adaptive immune system, required for T-lymphocyte differentiation and repertoire selection^[5,6], this gland was investigated to understand the effect of the maternal diet on fetal immune system development.

Methods: In this two factorial experiment design, pregnant beef cattle were fed with a low (L; 7%) or a high (H; 14%) protein diets from day 60 before artificial insemination to 23 days post-conception (dpc). At this stage half of the cows from each group were randomised to the contrary diet and the other half remained with the same diet until 98dpc, resulting in four dietary groups (LL, LH, HL and HH). The thymus from the fetuses from the four diet groups were dissected and the RNA extracted. The samples with the highest RNA quality were sent for RNA sequencing (2 male LL, 2 male HL, 2 male HH, 2 Female HH). RT-qPCR validation was undertaken with all samples within the study (Male n=14; Female n=14).

Results: Several genes that might be involved in development of the thymus and its function, such as AKAP9 and ROCK1, were altered in the 98dpc fetuses from dams fed low protein diets when compared with 98dpc fetuses from supplemented (high protein) dams. Genes involved in presentation of antigens to T-cells were also affected by sex.

Conclusion: Offspring undernourished *in utero* during peri-conception and first trimester of gestation might develop a weaker immune system compared with fetuses exposed to a high protein maternal intake. In addition, bovine males may have a stronger immune system than females.

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P111 Does parity make a difference in pregnancy outcomes in those conceived via assisted reproductive techniques (ART)?

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Background: With the increasing number of pregnancies occurring via the help of assisted reproductive techniques so do the consequences on both maternal and neonatal morbidity. ART pregnancies are considered at higher risk of perinatal morbidity and mortality compared to spontaneous conception. Whether parity contributes any role to those outcomes is not clear. This study aims to compare the outcomes between primiparous and multiparous women who conceived via ART. Results of this study could be utilised for counselling ART women appropriately.

Method: We prospectively collected data on all deliveries conceived via ART over five years. Cohort comparisons were made between primiparous and multiparous women. Specific outcome measures including induction of labour, method of and gestation at delivery, gestational diabetes (GDM), growth restriction (SGA), still births, shoulder dystocia, anal sphincter injury (OASIS), mean blood loss, post-partum haemorrhage (PPH) and neonatal morbidity and mortality were compared.

Results: A total of 11,881 deliveries over a 5 year period were included. Of those women conceived via ART were 549; 386 were primiparous and 163 were multiparous. It was more likely for primiparous women to have an instrumental delivery (23.8% vs 10.4%) and an emergency caesarean section (31.1% vs 16.6%). Multiparous women were noted to deliver at a significantly lower gestational age than primiparous (38.3/40 vs 39.2/40), and the likelihood of SGA was higher when primiparous (11.1% vs 4.3%). There were no differences in shoulder dystocia, OASIS, PPH and neonatal outcomes between the two groups.

Conclusions: The significant differences observed in our study were expected and are well known differences between primiparous and multiparous women in general. When reviewing more specific adverse outcomes they were not found to be different between the two groups. Based on our results we conclude that management of these women does not need to be altered on account of their parity.

P112 Donor sperm intrauterine insemination and the predictive factors influencing pregnancy outcomes

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Objective: To evaluate the pregnancy success rates in women undergoing donor sperm intrauterine insemination (IUI) and to assess the factors influencing the pregnancy outcomes.

Method: This retrospective audit of IUI cycles was carried out over two years (January 2016 to December 2017), using the local database and review of case notes. The patient population included same sex couples, single women and women where male partner had azoospermia. Various parameters including woman's age, BMI, indication, number of cycles, natural vs stimulated cycles, drugs and pregnancy outcomes were analysed.

Results: 87 women underwent IUI treatment (total of 349 cycles) and 38 out of the 87 achieved pregnancy (43.6%). Out of these 38 women, 32 had livebirth (36.7%) whilst 6 (6.8%) had miscarriage. 36% livebirth was seen equally distributed between the stimulated and unstimulated cycles (18% each). 49 women who did not conceive with IUI proceeded to IVF and 12 (24.4%) of them achieved positive pregnancy. Primary indication in 57% patients was same sex couple with primary infertility and they were equally distributed between both unstimulated and stimulated cycles. Women who conceived with the unstimulated cycles were predominantly aged < 35yrs, whereas most women aged >35yrs needed ovarian stimulation cycles. Gonadotropin was the drug used in 99% of the stimulation cycles. Women with primary infertility and BMI < 30 needed fewer than 3 cycles to achieve a successful pregnancy. There were only two twin pregnancies out of the 38 positive pregnancies (5%).

Conclusion: Our audit has shown that the pregnancy outcomes of unstimulated and stimulated cycles are comparable and hence has wider cost saving benefits. Although there is a predicted increased risk of multiple pregnancy with stimulated cycles, this was low (5%) in our audit. IUI is an effective, cheap and least invasive fertility procedure which should be offered as first choice to a selected cohort of patients.

P114 Estradiol is not required in the luteal phase of medicated frozen embryo transfers

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Background: Estradiol is required for endometrial proliferation in the early part of the menstrual cycle in order to "prime" the endometrium for subsequent implantation. Progesterone is mandatory for the luteal phase if implantation is to occur, but in animal models investigating implantation, oestradiol is omitted without detriment. However, in medicated (HRT) frozen embryo transfer (FET) treatment cycles, oestradiol is usually administered in the luteal phase as well. We report a case where oral oestradiol was mistakenly omitted from the time of vaginal progestogens, and yet implantation occurred, and the pregnancy continued unaffected.

Methods: A 46 year old woman receiving donor eggs used standard HRT protocol including oral Progynova (oestradiol) 6mg/day in the proliferative phase to coincide with the egg donor's stimulation. She started her Cyclogest (progestagen) pessaries on the day of the donor's egg retrieval. The embryos were cultured to day 5 (blastocyst) and an embryo transfer performed. However, it was only after the embryo transfer that she disclosed that she was only taking her Cyclogest pessaries. The Progynova was restarted immediately thereafter.

Results: Both medications were continued until her outcome day (10 days later) which revealed a positive pregnancy test. Both medications were continued until her early pregnancy scan which confirmed an ongoing singleton pregnancy. Both medications were discontinued at 12 weeks' gestation.

Discussion: Pregnancy was established in the absence of oestradiol in the early luteal phase. Although oestradiol is produced by the human corpus luteum, animal models investigating implantation do not require it. However, in medicated FET cycles, we traditionally use both oestradiol and progesterone, probably for fear of not given exogenous hormones. The inadvertent omission of oestradiol in the early luteal phase confirms that exogenous oestradiol is not required in the luteal phase (as per animal models) and can be omitted without fear of compromising implantation.

P115 What proportion of mosaic embryos are suitable for transfer when reviewed using the CoGEN position statement?

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A higher proportion of mosaic embryos are being detected following the introduction of Next Generation Sequencing for Preimplantation Genetic Testing for Aneuploidy (PGT-A), raising questions regarding the accuracy of trophectoderm samples when compared with the whole embryo and inner cell mass and casting doubt on the safety and effectiveness of transferring mosaic embryos. In 2016, CoGEN released its position statement indicating the transfer priority of mosaics. The purpose of this study was to assess the impact of this statement when applied to patient cases, by identifying what proportion of mosaic embryos would be considered for transfer.

Methods: Following the CoGEN position statement recommendation, we categorized mosaic embryos into 3 groups; high, medium and low priority for transfer:

- Highest priority: <40% of sample and only 1 chromosome involving chromosomes 1,3,4,5,6,8,9,10,11,12,17,19&20
- Medium priority: >40% of sample and only 1 chromosome involving chromosomes 1,3,4,5,6,8,9,10,11,12,17,19&20
- Lowest priority: mosaics involving 2 or more chromosomes or only 1 chromosome involving chromosomes 2,7,13,14,15,16,18,21,22,X&Y.

The categories were applied to those patients that had no euploid embryos but 1 or more mosaic embryo available after PGT-A.

Results and conclusion: 6614 PGT-A cases were reviewed. 1384 [20.9%] cases only had aneuploid embryos, 4538 [68.6%] cases had one or more euploid embryos and 692 [10.5%] case had no euploid and one or more mosaic embryo. The mosaic embryos in the no euploid, one or more mosaic group, when reviewed using priorities, resulted in:

- 111 [1.7%] of cases having at least one high priority mosaic available
- 184 [2.8%] of cases having no high priority but at least one medium priority mosaic available
- 397 [6.0%] of cases only having low priority mosaic embryos available.

Reviewing cases where these mosaics have been transferred, to establish implantation and pregnancy success rates in relation to the prioritization categories could provide valuable information for counselling patients undergoing PGT-A.

P116 A descriptive study of single centre 5 years' experience of embryo transfer under sedation

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Background: Embryo transfer under sedation is a recognised option for women in specific circumstances. In this study, we describe the indications and outcomes for women who have an ET under sedation.

Methods: A retrospective study of consecutive patients who underwent embryo transfer (ET) under sedation between January 2014 and August 2018 at a single centre.

Results: We analysed 5,572 cycles and identified 90 patients. The average age was 34.1 years old (range 24-43). 59 patients underwent ET fresh embryo (65.6%) and 31 patients had a FERC (34.4%). We identified three groups: (A) medical causes (46 patients) and (B) non medical causes (38 patients), in 6 patients a cause was not identified (C). In group A, we found history of previous difficult ET or difficult mock ET (37 patients), trachelectomy (5 patients), cervical stenosis (2 patients), female genital mutilation (1 patient) and one patient who had a spasm in the transvaginal oocyte retrieval. In group B, we included vaginismus (28 patients), patient choice (9 patients) and one patient who was virgo intacta.

Overall, 38 patients had a positive pregnancy test (42.2%), 18 (39.1%) patients in Group A and 16 (42%) in group B; in 4 patients in the group C. Of these pregnancies, 26 patients had a live birth (12 in Group A, 11 in Group B, 3 in Group C). 18 (69.2%) births were by caesarean section, forceps with 5 cases (19.2%), 2 vaginal deliveries (7.6%) and in one case data was not available.

Conclusions: Our data shows that embryo transfer under sedation is a viable option for women with a range of indications. The pregnancy outcomes are reassuring. However, the operative delivery rate is significantly higher than the national average.

Ovary

P119 How a general gynaecologist categorises and manages ovarian hyperstimulation syndrome

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Background: Severe ovarian hyperstimulation syndrome (OHSS) is a rare but potentially serious complication of IVF. Recently, media expressed concern that OHSS cases might be under-reported by IVF clinics to the Human Fertilisation and Embryology Authority (HFEA). However, most patients who present themselves to local hospitals, may be managed by clinicians who may have insufficient experience. Furthermore, discharge diagnosis and summary of management is traditionally reported by a junior member of staff. The aim of this study was to assess if the classification and management of cases admitted to hospital for presumed OHSS were in line with the Royal College of Obstetricians and Gynaecologists (RCOG) guideline 1.

Methods: The audit was carried out in a tertiary hospital affiliated with a large IVF clinic, between May 2008 and November 2017. The hospital and electronic notes of 44 inpatient cases, admitted for OHSS according to hospital discharge code, were scrutinised.

Results: This audit demonstrated that at least 70% of cases were mis-classified, 7% were uncategorised and 11% were not OHSS. 41% of patients had severe OHSS, 32% moderate, and 4% were mild once RCOG classification criteria were applied.

Two cases (5%) were critical but miscategorised at discharge. 25% required paracentesis. Low molecular weight heparin (LMWH) was only prescribed and administered in 75% of cases.

Conclusion: Our results provide evidence that the NHS coding system does not seem to be a reliable source for identifying cases of OHSS that need to be reported to HFEA. More needs to be done for general gynaecologists and junior staff to understand the classification and management of OHSS. Early discussion with the treating IVF clinic should be encouraged to help establish a correct diagnosis and aid appropriate management. This interaction will not only help management but can also help to improve accuracy of HFEA reporting.

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P120 Ultrasound monitoring during first-cycle treatment with clomifene citrate: A national survey of compliance with NICE

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Background: NICE CG156 advises that transvaginal ultrasonography (TVUS) should be used in the first cycle of treatment with clomifene citrate, to assess for multifollicular development and hence the risk of multiple pregnancy^[1]. This guidance is based on expert opinion rather than research evidence.

Method: We conducted a cross-sectional online and postal survey among UK-based consultant gynaecologists and fertility specialists, to explore compliance with this guideline.

Results: A total of 110 responses met the inclusion criteria. During first-cycle treatment with clomifene, 50.9% of respondents were not always using TVUS, and 21.8% never were. Clinicians who did not have immediate access to TVUS were significantly less likely to scan ($p < 0.01$). Other key factors influencing practice were, personal experience of the clinician, lack of an evidence base to support the guideline, and a willingness to accept the risk of multiple pregnancy. Several respondents questioned the value of scanning the first cycle only and highlighted that over-response may be seen in subsequent cycles.

Conclusion: This study confirms that there is variation in adherence to the guideline and uncertainty about the clinical need for scan monitoring. Further evidence to support or refute the guideline is required.

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P121 Functional characterization of the JAK/STAT and SOCS pathway in human granulosa cells

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Aims and objectives: Oocyte-granulosa cell signalling interactions facilitate and encourage proper maturation events during ovarian development. A failure to control the signals stimulating oocyte development results in infertility. In patients with Primary Ovarian Insufficiency, it is not clear how oocytes within primordial follicles are activated. The aim of this study was to determine if members of the JAK/STAT and SOCS pathway are expressed in human granulosa cells, and if they play a functional role in human primordial follicle signalling.

Content: COV434 cells, a human granulosa cell line, were used as an in vitro model of human granulosa cells. Quantitative PCR, immunoblotting and immunostaining of COV434 cells were performed for JAK1, STAT1, STAT3 and CISH. Chemical inhibition of the JAK1/STAT pathway in COV434 cells was achieved using the potent JAK inhibitor Ruxolitinib. To validate our model, immunostaining was performed on human ovarian sections from pre-menopausal women aged between 34-37 years of age.

Relevance/impact: The JAK/STAT and SOCS pathway is present and functional in human granulosa cells, indicating the potential to directly target the pathway as a method of regulating the ovarian reserve and female fertility.

Outcomes: JAK1, STAT1, STAT3, SOCS4 and CISH are expressed in human granulosa cells, at similar levels. Ruxolitinib treatment of COV434 cells resulted in increased STAT3 mRNA expression ($p \leq 0.05$) and increased pSTAT1 protein ($p \leq 0.01$). The expression of JAK1, STAT1, STAT3 and SOCS4 was primarily detected in the granulosa cells in human ovarian sections.

Discussion: Our results indicate that JAK/STAT and SOCS members are present with the human ovary, specifically within the granulosa cells. This data may provide valuable insights into how the somatic support cells contribute to ovarian development and may benefit the development of potential diagnostic and therapeutic methods for patients suffering from ovarian-related infertility.

P122 Going, gonad, gone: A retrospective review of the management of women with suspected ovarian torsion

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Introduction: Ovarian torsion is a gynaecological emergency. Although difficult, prompt management is critical to avoid loss of ovarian function. Historically, treatment involved oophorectomy, but de-torsion has since been demonstrated to restore function, thereby preserving fertility^[1]. Delaying surgery by more than 48hrs however reduces the possibility of ovarian salvage^[2].

Aims: To investigate how women with suspected ovarian torsion present, how they are managed and the overall timeliness of the clinical episode.

Methods: We reviewed all women who underwent surgery for suspected ovarian torsion between 1/4/16 and 31/3/18. Alongside presenting symptoms, examination findings and investigation results, we recorded the following times: symptom onset; hospital presentation; and knife-to-skin. We also documented the surgical procedure(s) performed and intra-operative findings.

Results: 31 women (mean age 29.4+/-7.1yrs) underwent surgery for suspected ovarian torsion. The median time between symptom onset and hospital presentation was 21.6hrs (IQR:7.9-78.1hrs) and between presentation and knife-to-skin was 26.2hrs (IQR:12.3-47.5hrs). Surgery occurred more than 48hrs after symptom onset in 21(67.7%) women. Intraoperatively, 20(64.5%) women had torsion confirmed. There were no significant differences in presenting symptoms, examination findings, investigation results or time intervals between those with and without confirmed torsion. 13(65%) torsed ovaries were necrotic. There was no difference between symptom onset and knife-to-skin time for women with a viable and necrotic ovary (74.3hrs,IQR:33.8-132.3hrs versus 74.8hrs,IQR:36.5-114.5,p=0.968). All necrotic ovaries were removed.

Conclusion: In women with acute pelvic pain, differentiating between those with and without ovarian torsion pre-operatively is difficult. Furthermore, women delay seeking medical attention and hence most women with torsion have a necrotic ovary by the time of surgery and are (perhaps unnecessarily) undergoing oophorectomy which is detrimental for the subsequent fertility of these predominantly young women. It is imperative that those involved in the management of these women have a low threshold for timely surgical intervention. Guidelines, such as those that exist for testicular torsion, may be beneficial.

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P124 Patterns of anti-Müllerian hormone and vitamin D receptor expression at different phases of oestrous cycle in sheep ovaries

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With respect to oogenesis and ovarian follicle development, vitamin D is known to antagonize FSH, primordial follicles recruitment and Anti-müllerian hormone (AMH) secretion. AMH is a glycoprotein copiously secreted by primary, secondary and small antral follicles. Progression from one phase of oestrous cycle (OC) to another is associated with characteristic populations of different follicular types.

Fifteen multiparous cycling Welsh Mountain ewes were used to evaluate the trend of AMH and vitamin D receptor (VDR) expression across OC. The ewes had their OC synchronized using medoxyprogesterone acetate intravaginal sponges and intramuscular injection of 300-I-U PMSG. On day 8 of OC, ovaries were randomly collected from 3 ewes and this constituted the Mid-luteal group (MLG). On day 11 of OC, 125-ug of PGF2alpha was given and ovaries randomly collected after 32h [pre-LH peak group (Pre-LPG)], 39h [LH peak group (LPG)], 46h [post-LH peak group (Post-LPG)], and 84h [early luteal group (ELG)] respectively (n=3; time point).

Concentration of LH in plasma samples was determined by radioimmunoassay. Immunohistochemistry, Western Blotting (WB) and RT-PCR were used to detect and quantify the expression of AMH and VDR. Immunostaining detected AMH in granulosa cells of developing follicles. Staining intensity was greatest in Pre-LPG ovaries and least in MLG ovaries. These results matched WB analysis of AMH protein, showing the strongest signal in pre-LPG ovaries. Similarly, AMH mRNA expression was highest (p<0.05) in Pre-LPG samples. Post-LPG and ELG ovaries had significantly (p<0.05) higher VDR mRNA expression. The MLG had the least (p<0.05) expression of both AMH and VDR.

The results indicate that with the exception of the quiescent phase represented by MLG, VDR and AMH expressions are inversely related across OC. The pre-LPG which represents the proestrus phase of OC could have expressed comparatively higher AMH possibly due to predominance of recruited growing follicles.

P125 Regulation of anti-Müllerian hormone (AMH) expression: correlation with vitamin D and oestrogen receptor

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Background: Vitamin D has a fundamental role in reproduction and its actions are mediated exclusively by its receptor (VDR). Anti-Müllerian hormone (AMH) also has an important function in normal and disordered ovarian folliculogenesis associated with PCOS. The discovery of a VD response element on the AMH promoter indicates that VD regulates AMH expression^[1]. There is growing evidence to support the down-regulation of AMH expression by oestradiol (E2), primarily via alteration of the ratio of oestrogen receptors (ER) α and β (2)

Aims: To investigate the relationship between the expression of AMH, VDR and ERs in mouse ovarian follicles and using the human KGN granulosa cell-line.

Methods: KGN cells were treated with testosterone (500nM) $\pm$ forskolin (25 μ M) $\pm$ PHTPP - a selective ER β antagonist (at 10-6, 10-7 & 10-8M) for 48h. The mRNA expression of ER α , ER β and AMH was measured using qPCR with RPL7 as the normalizer (n=4-5). Using immunohistochemistry (IHC), the expression of AMH, VDR and ER β in mouse ovaries (n=3) were analysed.

Results & conclusions: Forskolin increased ER α :ER β (150-fold) in KGNs, although no increase in AMH mRNA expression was seen. PHTPP increased ER α :ER β (40-fold at 10-6M) in a dose-dependent manner, with a corresponding 7-fold increase in AMH mRNA expression. AMH protein levels rose throughout the early stages of folliculogenesis with maximal expression in late pre-antral and early antral follicles. VDR expression was strongest in pre-ovulatory follicles but no clear relationship between VDR and AMH expression was seen. There was an inverse relationship between AMH and ER β expression, with later stage follicles expressing less AMH but more ER β . The increase in ER α :ER β expression by forskolin did not increase AMH expression, in contrast to treatment with the selective ER β antagonist PHTPP. One possible explanation is that cross-talk from other pathways activated by the forskolin-induced increase in cAMP, could inhibit AMH expression.

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P126 Effects of (E.coli) LPS on the luteal angiogenesis and progesterone production in vitro

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Introduction: Uterine inflammation caused by Gram-negative bacteria in dairy cows inhibits ovarian function and decreases fertility. This study investigated the effects of the bacterial endotoxin, lipopolysaccharide (LPS) on the luteal steroidogenic and angiogenic characteristics in vitro.

Methods: Luteal cells (n=4 cultures) were enzymatically dispersed from bovine corpora lutea (very early) and were incubated at 39°C for 5 days. From day 1 of culture, cells were treated with LPS (0, 0.01 or 1 μ g/ml) in the presence of angiogenic stimulation (1ng/ml fibroblast growth factor-2 and 1ng/ml vascular endothelial growth factor-A. Spent media from days 3 and 5 was analysed for progesterone by ELISA. On day 5, endothelial cells were immunostained for Von Willebrand factor and network formation quantified by image analysis. Next, luteal cells (n=4 culture) were treated similarly with LPS with dual immunofluorescence staining for Ki67 (proliferation) and caspase (apoptosis) with isolectin B4 (endothelial) being performed. Protein was extracted from a subset of cells were lysed for Western blots analysis of Steroidogenic acute regulatory protein; 3 β -Hydroxysteroid dehydrogenase; Cytochrome p450 side-chain cleavage and smooth muscle actin protein expression.

Results: LPS decreased the total area of endothelial cell networks, number of endothelial cell islands and branch points as well as degree of branching (all p<0.01). Progesterone production was unaffected by LPS treatment as was 3 β -Hydroxysteroid dehydrogenase, Cytochrome p450 side-chain cleavage, and Steroidogenic acute regulatory protein expression (all p>0.05). LPS decreased the proliferation index (Ki67-positive) of endothelial cells by 2-fold (p<0.01). Conversely, LPS increased endothelial cell apoptotic index (caspase 3-positive) in a dose dependent manner (p<0.001). Additionally, LPS had reduced the expression of smooth muscle actin protein (P<0.01), a mural cell marker.

Conclusion: Lipopolysaccharide suppressed luteal endothelial cell network formation in a dose dependent manner in vitro, potentially by increasing apoptosis and suppressing proliferation of luteal endothelial cells. In addition, LPS is likely to adversely affect the number of vascular mural cells. At the same time, LPS had no direct effect on progesterone production and expression of steroidogenic proteins.

P127 Immunolocalization of PPAR α and PPAR β / δ in the bovine corpus luteum

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Objectives: Peroxisome proliferator-activated receptor (PPAR): α , β/δ and γ are involved in the local regulation of reproductive tract including ovulation, implantation process and luteolysis. Up to date three PPAR isoforms were present in the bovine endometrium throughout the estrous cycle^[1]. Furthermore, the presence of PPAR γ was examined in the bovine CL^[2]. Therefore, we hypothesized that other PPAR isoforms (α and β/δ) are present in the bovine CL dependently on the lifespan of CL. The aim of this study was to determine the immunolocalization of PPAR α and PPAR β/δ in the bovine CL during different phases of the estrous cycle.

Methods: Corpora lutea (CLs) were dissected from *post mortem* cows as following: early luteal I (n=4), early luteal II (n=4), mid luteal (n=4), late luteal (n=4), and CL regression (n=4) phases of the estrous cycle. The immunolocalization of PPAR α and PPAR β/δ was evaluated by immunohistochemical staining.

Results: Immunohistochemistry revealed the expression of PPAR α and PPAR β/δ in the examined bovine CLs. Both isoforms α and β/δ were detected and localized in the perinuclear cytoplasm and nuclei of luteal cells at early-, mid- and late- luteal phases of the estrous cycle, whereas the lack of immunoreactivity in the nuclei of luteal cells was observed at CL regression phase.

Conclusions: The results provided evidence for the presence of PPAR isoforms: α and β/δ in the bovine CL during different stages of the estrous cycle suggesting that PPARs may regulate genes required for development, maintenance and regression of CL. This research was supported by the National Science Centre 2014/15/N/NZ9/02428

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P128 Fibroblast growth factor 2 upregulates dual specificity phosphatases (DUSP) in ovine granulosa cells

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The DUSPs comprise a large family of over 60 enzymes that dephosphorylate their target substrate on serine/threonine and tyrosine residues. Twenty-three DUSPs are involved in the MAPK signaling pathway^[1], which is a major pathway activated by fibroblast growth factors (FGF). Previous data from our laboratory show that FGF18 causes atresia and FGF2 does not^[2]. FGF2 signals by rapid and transient increases in MAPK3/1 phosphorylation whereas FGF18 causes a low-level sustained rise in MAPK3/1 phosphorylation. We hypothesized that this different pattern of MAPK signalling results from different regulation of DUSP expression.

The aim of the present study was to describe MKP/A-DUSP expression in ovine granulosa cells and their regulation by FGF. Sheep ovaries were collected from a local slaughterhouse and granulosa cells were harvested from small (1-3mm), medium (3-5mm) and large (>5mm) antral follicles for RNA extraction. Granulosa cells from medium and large antral follicles were isolated and cultured in serum-free conditions. On day 5 of culture, cells were stimulated with FGF2 or FGF18 at different times (1, 2, 4, 8h) or doses (1, 10, 100ng). Abundance of mRNA encoding DUSPs was assayed by RT-qPCR. Sixteen out of 23 DUSPs were expressed in freshly harvested granulosa cells irrespective of follicle size. *In vitro*, treatment with FGF2 caused a rapid and transient increase of *DUSP1* mRNA levels that returned to control levels within 2h. Treatment with FGF2 increased *DUSP5* mRNA abundance at all time points, and *DUSP6* mRNA levels increased only at the later time point (8h).

All doses tested increased mRNA levels to the same degree. In contrast, treatment with FGF18 did not alter DUSP mRNA levels at any time or dose tested. We conclude that the difference in MAPK signal elicited by FGF2 and FGF18 may be caused in part by changes in DUSP expression.

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P129 The effect of season on the incidence of multiple ovulation in UK dairy cows

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In many countries seasonal climatic conditions have an important influence on many aspects of reproductive function. The aim of this study was to determine if, under the temperate UK climate, ovulation rate in dairy cows was influenced by season.

Ovaries from a total of 1591 cows were collected from a single abattoir over 2 consecutive years at 4 seasonal time points (winter and summer solstices and spring and autumn equinoxes). Corpora lutea and follicle populations were quantified and luteal progesterone content measured. Incidence of multiple ovulation was analysed by logistic regression with binomial distribution and follicle population and luteal progesterone content analyses by linear mixed model.

The overall incidence of multiple ovulation was 9.6% and was higher ($P < 0.05$) during summer (12.2%) and autumn (10.9%) than during winter (6.9%) with incidence in spring (8.4%) not significantly different from other seasons. While the number and distribution of follicle populations was not influenced by season, the number of large follicles ($> 7\text{mm}$) was higher ($P < 0.05$) in multiple (2.53) compared to single (2.18) ovulating cows. Individual corpus luteum weight was lower in multiple ovulating cows (2.5g vs 3.5g; $P < 0.001$) while total weight of luteal tissue was higher (5.1g; $P < 0.001$).

Overall, luteal weight was higher ($P < 0.001$) in autumn than in spring (3.3 vs 2.8g) with winter and summer not significantly different from other seasons. Total luteal progesterone content was not affected by season or ovulation status. These results demonstrate an elevated incidence of multiple ovulation during the summer with multiple ovulation associated with an increased number of large follicles on the ovaries and increased total luteal mass.

P130 Isolation of different bovine luteal cell fractions using fluorescence-activated cell sorting

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The corpus luteum (CL) is a highly heterogeneous tissue (containing steroidogenic, endothelial, immune and other cell types) whose structure and cellular composition dynamically changes in response to hormonal changes throughout the oestrus cycle. Isolation of purified cell populations from luteal tissue extracts for later characterization has been difficult. The goal of this study was to establish techniques for selective isolation of steroidogenic and endothelial cell fraction from bovine CL using Fluorescence-Activated Cell Sorting (FACS), and to characterise the expression of miRNAs previously identified by^[1] us to be critical for luteal development.

Immunofluorescence (IF) and flow cytometry analyses showed that CD144, a surface marker, and Nile Red (NR), a lipophilic stain, specifically located to endothelial and steroidogenic cells, respectively, in luteal sections. Using these markers, we isolated these two cell fractions from bovine luteal extracts using FACS. We used RT-qPCR to confirm the identity of the cell fractions obtained and found that the CD144 was predominantly expressed in the CD144+ cells while HSD3B1 was expressed mostly in NR+ cells. Moreover, expression of miR-126, an endothelia-specific marker, was restricted to the CD144+ fraction, further validating our approach. Finally, we analysed the expression of two different miRNA clusters which we have previously shown to be involved in luteal survival and steroidogenesis (miR-212-132 and miR-183-92-182) and found that while miR-212 and miR-132 were expressed at similar levels in both CD144+ and NR+ luteal fractions, both miR-183 and miR-96 were expressed at much higher levels in CD144+ than in NR+ cells.

In summary, we have shown that different cell fractions can be effectively isolated from corpora lutea using FACS allowing targeted analyses of specific cell populations involved in luteal development and function.

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P131 TGFβ/SMAD3 pathway regulates transzonal projection (TZP) formation in growing follicles of the mouse ovary

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In the mammalian ovary, each oocyte is surrounded by a layer of somatic cells, called granulosa cells (GCs), creating a functional unit termed the ovarian follicle. Shortly after the initiation of follicular development, a glycoprotein layer called zona pellucida (ZP) is generated that physically separates the growing oocyte from the proliferating granulosa cells that surround it. Since GC-oocyte contact-dependent communication is essential for oocyte development, GCs generate filopodia, termed transzonal projections (TZPs), that penetrate the ZP and establish contact with the oocyte plasma membrane.

Previous results have shown that growth-differentiation factor (GDF) 9, a TGFβ ligand secreted by the oocyte, increases the steady-state levels of Myo10 and Fscn1, which encode proteins implicated in filopodial assembly as well as the number of TZPs in granulosa-oocyte cell complexes (GOCs). Therefore, we sought to investigate whether the intracellular TGFβ pathway mediators, SMAD3 and SMAD4, regulate the expression of Myo10 and Fscn1 genes.

Quantitative RT-PCR of mouse ovaries at day-4 and day-12 of age (enriched in non-growing and growing follicles, respectively) showed a significant increase in the relative mRNA levels of Fscn1 and Myo10 mRNAs during growth. Consistent with this, immunofluorescent staining of ovarian histological sections localised SMAD3, Fscn1 and MYO10 in GCs of follicles

from primary stage onwards. Moreover, ChIP-qPCR revealed that SMAD3, although not SMAD4, was bound to the promoter region of *Fscn1* and *Myo10*.

Our results suggest a direct regulation of *Fscn1* and *Myo10* genes by the canonical TGF β /SMAD3 pathway in GCs of growing follicles. Current work focuses on establishing an inducible system to deplete SMAD3 and SMAD4, to study the effect of impaired SMAD signalling on expression of *Fscn1* and *Myo10*, TZP number, and follicular growth. The results may reveal the molecular mechanism of TZP formation and identify a new role for SMAD signalling during early follicle development.

P132 Effect of DHEA supplementation on ovarian function during the perimenopause

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There is growing evidence that dehydroepiandrosterone (DHEA) supplementation in women with diminished ovarian function can improve oocyte quality. However, it is unclear if similar beneficial effects of DHEA supplementation can be obtained during the perimenopause, when the number of ovarian follicles is markedly lower. To address this question we examined the impact of 2.5-months of daily oral DHEA supplementation (5 mg) on the number of ovarian follicles and on the concentrations of anti-Müllerian hormone (AMH) in perimenopausal rhesus macaques. Like women, these long-lived nonhuman primates have ~28-day menstrual cycles and eventually undergo menopause. Furthermore, they show similar age-related neuroendocrine changes including a marked decrease in circulating DHEA concentrations.

Our experimental design involved the following three groups of animals (N = 6 per group): Young adult (mean age = 11.6 years), Old control (mean age = 23.1 years), and Old DHEA-treated (mean age = 23.5 years). Histological examination of the ovaries revealed a significant (P<0.05) age-related decrease in the mean number of pre-antral follicles but no obvious beneficial effect of DHEA supplementation. Similarly, AMH concentrations within the ovaries and circulation, assessed by Western analysis and ELISA respectively, showed a significant (P<0.05) age-related decrease but no obvious beneficial effect of DHEA supplementation.

Taken together, the results indicate that short-term DHEA supplementation alone is insufficient to enhance ovarian function during the perimenopause and they complement findings from our earlier studies, which showed no obvious benefit of DHEA supplementation on cognitive function. On the other hand, it remains plausible that chronic DHEA supplementation, earlier DHEA intervention, and supra-physiological DHEA doses may have more therapeutic potential.

P133 Optimising the derivation of granulosa cells from mouse pluripotent stem cells

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Background: Granulosa cells (GCs) regulate the survival, development and function of oocytes, but knowledge of GC origin and development is limited. GC differentiation from pluripotent stem cells (PSCs) has been reported, but is inefficient, and the functional capacity of these cells remains unclear. This project aims to improve the derivation of GCs from PSCs, by directing differentiation towards GC lineage using step-wise growth factor treatment to recapitulate embryonic gonad development.

Methods: Pluripotent P19 embryoid carcinoma cells were induced to form mesoderm through culture with 0.5% DMSO for 8 days (n=3). E14Tg2a mouse embryonic stem cells (mESCs) were differentiated for 12 days in monolayer culture in the absence of LIF (n=3). RNA was extracted at regular time points. Gene expression was analysed using RT-qPCR, with primers selected to assess stages of mESC differentiation/GC formation (pluripotency, mesoderm, gonad).

Results: *Brachyury* and *Wt1* were upregulated, and pluripotency marker *Oct4* downregulated, during differentiation of P19 cells to mesoderm, supporting the use of these markers to track early differentiation. In differentiating mESC cultures, *Oct4* expression decreased to 25.8±5.6% that of undifferentiated day (d) 0 samples by d8, and remained low at subsequent time points. By d8, *StAR* expression had decreased to 40.3±4.6% that of d0 levels, but subsequently increased to 89.3±12% by d10 of differentiation. *Cyp19a1* transcripts were only detected at d10 and d12, but levels varied between cultures. *Amh* was stably expressed across all time points examined.

Conclusions: Upregulation of steroidogenic-associated genes (*Cyp19a1* and *StAR*) at d10-12 of mESC differentiation suggests the emergence of an ovarian somatic cell-like population, and is consistent with reports of ovarian follicle-like structures forming from differentiating mESCs at similar time points. These data provide a baseline against which the impact of exogenous growth factor supplementation on the efficiency of GC derivation from mESCs can be investigated.

P134 Modulation of the Notch system in response to Wnt inhibition induces restoration of the rat luteal function

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The aim of this study was to investigate whether the Notch pathway is modulated in response to the down-regulation of the Wnt/B-Catenin system in corpora lutea (CLs) from superovulated rats. To this end, we analyzed the effect of *in vitro* CL Wnt/B-Catenin inhibition on the expression of Notch members and on luteal function. Mechanically isolated rat CLs were cultured with ICG-001, a Wnt/B-Catenin inhibitor.

In this system, Wnt/B-Catenin inhibition reduced progesterone production and decreased StAR protein levels. Besides, Wnt/B-catenin inhibition stimulated the Notch system, evidenced by an increase in Hes1 expression, and promoted the expression of selected Notch family members.

At long incubation times, StAR levels and progesterone concentration reached the control values, effects probably mediated by the Notch pathway. These results provide the first evidence of a compensatory mechanism between Wnt/B-Catenin signalling and the Notch system, which contributes to the homeostasis of luteal cells.

P135 The effect of Benzo[a]pyrene on proliferation and number of germ cells in the developing mouse ovary

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Background: Benzo[a]pyrene (B[a]P), a polycyclic aromatic hydrocarbon found in cigarette smoke, grilled foods and coal tar, has been found to decrease the number of primordial follicles in the developing mouse ovary^[1], however the exact mechanism of action is unknown. By reducing the size of the ovarian reserve, B[a]P negatively affects future fertility. Investigating this process is of particular importance as 10-17% of pregnant women in the UK are known to be smokers. The aim of this project was to establish whether B[a]P's effect on newly formed follicles might be via a disruption of germ cell proliferation.

Methods: Pregnant time-mated female mice were sacrificed at embryonic day 12.5 (E12.5) and E13.5. Embryonic mouse ovaries were cultured *in vitro* on agar blocks for 24 hours with B[a]P and BrdU, a cell proliferation marker, added for the duration of culture. Immunohistochemistry was then carried out on the cultured ovaries for BrdU, to identify cells that had undergone proliferation, and DDX4, to identify germ cells; DAPI was used as a general cell marker. Analyses determined the total number of germ cells in the ovary and the percentage of these that had undergone proliferation during the culture period.

Results and discussion: B[a]P had no effect on germ cell proliferation in the developing mouse ovary at either E12.5 (treatment group: 90.1% vs control: 88.3%; $p=0.3919$, $n=12$) or E13.5 (treatment group: 67.5% vs control: 70.1%; $p=0.7292$, $n=13$). Additionally, B[a]P did not affect the total number of germ cells either at E12.5 (treatment group: 2323 vs control: 1499; $p=0.0662$, $n=12$) or E13.5 (treatment group: 3767 vs control: 3538; $p=0.7016$, $n=13$). We conclude that any effect of B[a]P on the developing mouse ovary is not via germ cell proliferation immediately prior to follicle formation.

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P136 Does *in vitro* environment of cultured human cryopreserved ovarian tissue affect follicle development and health?

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Background: *In vitro* growth of ovarian follicles has been suggested as an alternative to autotransplantation for patients for whom other fertility preservation methods might be contraindicated. Complete human oocyte growth was recently achieved using a multi-step culture model, however the development of follicles within cortical strips remains limited. The aim of this study was to determine whether culture condition affected human follicle health and development.

Methods: Cryopreserved ovarian cortical strips from three cancer patients (17-25 years) were thawed, sliced and cultured for 6 days. Three physical conditions were tested: high medium volume (1 mL) with a polycarbonate membrane, low medium volume (300 μ L), or high medium volume in a gas-permeable plate. Two media were tested: McCoy's 5A and α MEM. Follicle development and health was assessed based on histology. Generalised linear mixed modelling and logistic regression modelling were used to determine the effect of culture condition on development and health, adjusted for patient and tissue volume.

Results: A total of 5,836 follicles were analysed. There were significantly fewer primary follicles in non-cultured tissue compared to primordial or transitional follicles ($p < 0.0001$). In cultured tissue (McCoy's, high volume; MH) there were a greater number of transitional ($p < 0.01$) or and primary ($p < 0.0001$) follicles compared to primordial follicles. Follicle development was largely unaffected by culture condition. Fewer transitional follicles were observed in McCoy's low volume compared to MH ($p < 0.01$). Compared to non-cultured tissue, all cultured tissue exhibited a less healthy morphology. There

was no difference in primordial follicle health for the different culture conditions. A transitional or primary follicle was significantly more likely to be healthy when cultured under low volume conditions irrespective of medium, compared to MH.

Conclusion: Our results suggest medium volume, rather than composition is critical for optimizing health of human follicles cultured *in vitro*.

P137 Polycystic ovarian syndrome - presentation and management outcome in secondary care

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Introduction: Polycystic ovarian syndrome (PCOS) is the commonest endocrine disturbance affecting women of reproductive age group and comprises a heterogeneous collection of signs and symptoms. According to the Rotterdam criteria, 2 out of 3 features are needed to diagnose PCOS. These are oligo-ovulation and/or anovulation, hyperandrogenism (clinical and/or biochemical) and polycystic ovaries on Ultrasound scan, with the exclusion of other aetiologies.

Aims/objectives:

- To look at the prevalence and presenting features of PCOS in our patient population
- To look at the management outcome in our unit.

Method: Retrospective study over 1 year Data from electronic database Simple statistical methods to analyse the data.

Results: Total 515 patients in the Fertility clinic. PCOS according to Rotterdam criteria diagnosed in 119 (23%). Scan findings of only PCO 176 (34%). Obese PCOS 66 (55%), menstrual irregularity 71 (60%), hyperandrogenism 35 (29%). For obese group (n=66), lifestyle modification helped to resume cycle and conception in 39 (59%). For 41% of obese (27), 10 (15%) conceived with either Clomiphene citrate (CC) or Assisted Reproductive Techniques (ART) or both. Non-obese PCOS 53 (45%), 25 had Laparoscopic ovarian drilling (LOD) due to CC resistance/failure with or without other pathologies like suspected endometriosis/tubal pathology. The ovulatory PCOS were not subjected to CC. Out of LOD (n=25), 11 (44%) conceived (spontaneous or ART).

Conclusions: Using proper diagnostic criteria helped in the identification of PCOS patients and stepwise management has shown a positive impact on the outcome in terms of symptom control and conception for these women. RCOG has recommended lifestyle changes as the first line of treatment for women with PCOS. Both RCOG and NICE have highlighted the role of LOD in ovulation induction, minimising the risk of multiple pregnancy and ovarian hyperstimulation.

Uterus/Tract

P138 A case series highlighting endometrial hyperplasia as an important issue in the young fertility patient with polycystic ovarian syndrome

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Introduction: Polycystic ovarian syndrome (PCOS) is associated with high risk of endometrial hyperplasia (EH) and malignancy^[1]. EH is caused by unopposed oestrogenic states which over-stimulate endometrial cells causing hypertrophy and endometrial dysfunction. Uterine receptivity and implantation is known to be reduced in PCOS patients^[2,3] but will be further hindered by development of hyperplasia and reduce success of fertility treatment. This case series details five young patients with a current or past history of irregular menstrual cycles undergoing fertility investigations and treatment that were then diagnosed with simple and complex hyperplasia.

Case series: Patients were diagnosed as PCOS using the 2003 Rotterdam consensus diagnostic criteria. All were under the age of 35 with a period of infertility ranging from 1 to 12 years. They had differing gynaecological history and bleeding patterns ranging from regular 28 day cycles (Case C) to continuous vaginal bleeding for one year (Case A). Case A was the only patient with a raised BMI >35. Interestingly, case C, D and E had no features on transvaginal ultrasound suggesting hyperplasia, but Case C was diagnosed with hysteroscopy and biopsy as per protocol after two failed IVF cycles. Case B underwent endometrial sampling after failing suppression with GnRH agonist at the start of an IVF cycle. All were treated with oral progestogens.

Discussion: Cycle length of 84 days and/or endometrial thickness >7mm indicates PCOS patients at risk of hyperplasia(4-5). This series highlights the variation in presentations and potential failures in identifying the PCOS patients at risk when reliant on those parameters alone. EH is a concern for patients with oligo-/anovulation due to the risk of recurrent implantation failure with treatment, delay to fertility treatment and the malignant potential. This case series raises the question of empirical endometrial sampling for all fertility patients with irregular cycles regardless of scan findings.

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P139 Assessment of tissue viability following controlled-rate cryopreservation of ovine uteri

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Uterine transplantation is an option for uterine factor infertility (UFI) patients^[1]. Cold ischaemic storage is the current uterine preservation strategy between retrieval and transplantation^[1]. Thus, cryopreservation offers a possibility of storage allowing a longer window between surgical procedures, whilst tissue viability and functionality^[2]. This study explored uterine cryopreservation effects on tissue viability (cell cytotoxicity and endometrial prostaglandin production) and myometrial contractility.

Twenty-six uterine horns were infused (0.5ml/min) with cold heparinised-solution (Hep) followed by CPA (0.1M Sucrose, 1.5M DMSO, 10% FBS in Leibovitz-L15) for 40 min (SF40, n=10) or 60 min (SF60, n=10), and cryopreserved using a controlled-rate freezer (Planer Ltd.). Remaining uterine horns (CT, n=6) received Hep infusion only. All uterine horns (SF post-thawing, and CT post-Hep) received a bolus dose of oxytocin (OT; 1M), followed by endometrial tissue culture for 48h. Tissue damage was evaluated by lactate dehydrogenase release (LDH; Roche) and endometrial viability by prostaglandin F2alpha production (PGF2a; Enzo). Uterine contraction was observed in all groups following OT, with SF40 and SF60 showing a reduced myometrial contractility (P<0.05). Similarly, PGF2a production by cultured endometrial explants was observed after 24h and 48h, with cryopreservation reducing nearly 30% compared to CT (P<0.05).

LDH production was highest (P<0.05) in endometrial tissue compared to myometrium or vascular tissue. While no difference was observed between SF40 and SF60 (P>0.05), LDH release was significantly lower in CT (P<0.05). In conclusion, following cryopreservation, uterine tissue was able to contract following OT injection and produce endometrial PGF2a after 48h in culture. However, both activities were reduced due to an increase in tissue damage. These results are encouraging and indicate that refinement of cryopreservation protocols (e.g. infusion time, freezing rate) may improve the outcome and allow uterine tissue banking as a resource for uterine transplantation for UFI patients.

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P140 Dissecting the molecular mechanisms of long non-coding RNA function in X chromosome inactivation across mammalian gestation evolution

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Sex chromosome dosage compensation in eutherian mammals is mediated via the long non-coding RNA Xist. Despite Xist's conservation in eutheria, most studies have focused on its role in the mouse or human stem cells. The aim of this study was to determine if Xist and its direct protein partners are conserved in eutherian mammals with divergent reproductive morphologies.

Murine, human, bovine and porcine RNA sequences of Xist and known protein interactors in the mouse (Spen, Lbr, Ciz1, hnRNPU, hnRNPK, Rbm15 and Wtap) were aligned using Multiple Alignment Fast Fourier Transform (MAFFT). Concurrent expression of XIST and its candidate protein partners in terminally differentiated tissue (endometrial-derived) was examined by RT-qPCR. Xist RNA conservation between mouse, human, cattle and pig sequences ranged at specific short nucleotide stretches (51-90%) but across the sequence as a whole, conservation was modest (61-63%). Between all four species, some mouse Xist interactors were modestly conserved at the peptide sequence such as Ciz1 (68-82%) and Lbr (79-87%), however most were highly conserved (Spen (80-90%), Wtap (94-96%), Rbm15 (94-98%), hnRNPU (96-98%) and hnRNPK (98-100%)).

RNA recognition motifs (Spen) and K homology domains (hnRNPK) displayed 99% similarity within all species. Wtap and Lbr do not possess canonical RNA-binding domains. The RNA levels of Xist and its putative protein partners (Spen, Lbr, Ciz1, hnRNPU, hnRNPK, Rbm15 and Wtap) were expressed comparably in cattle. Mouse Ciz1 and Lbr mRNA levels were lower compared to Xist (p<0.05) whereas hnRNPU levels were higher (p<0.05). ISHIKAWA-expressed XIST RNA was lower compared to all putative partners tested (p<0.01).

High conservation at specific regions of Xist and its putative interactors tied to their coordinate presence in differentiated tissue supports that they may interact in mice, humans, cattle and pigs. Future work will focus on Xist pull-down and mass-spectrometry to biochemically confirm interactions in our species of interest.

P141 Study of the impact of selective progesterone receptor modulators upon apoptosis in the human endometrium

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Background: Heavy menstrual bleeding (HMB) affects 1 in 4 women of reproductive age in the UK. Despite the high prevalence of HMB current treatments are often ineffective or have significant side-effects. Selective progesterone receptor modulators (SPRMs), provide an alternative pharmacological approach. SPRMs exert mixed agonistic and antagonistic effects on the progesterone receptor and reduce menstrual bleeding in women with uterine fibroids. The mechanism of action of SPRMs is not fully understood. SPRM administration increases apoptosis and reduces proliferation in uterine fibroids. The impact of SPRM administration on the uterine endometrium has received scant attention. The current study has investigated how endometrial apoptosis is modulated following exposure to the SPRM, ulipristal acetate (UPA). It is hypothesised that SPRM administration alters apoptosis in human endometrial tissue.

Aims: This project aimed to describe presence and location of specific apoptotic markers (Caspase-3, BAX and BCL-2) in the endometrium following SPRM (UPA) administration.

Methods: Well documented endometrial tissue was collected from patients in NHS Lothian during the UCON study (Ulipristal acetate versus conventional management of heavy menstrual bleeding - Trial number: EUDRACT 2014-003408-65. REC: 14/LO/16020; written informed consent provided by all women) were available for study. Endometrial biopsies were collected (i) before UPA treatment, (ii) after 6 months of UPA treatment and, (iii) at 12 months, following cessation of UPA and a menstrual bleed. To investigate presence of apoptosis in tissue samples, relative mRNA expression of several apoptotic markers (Caspase-3, BAX and BCL-2) was studied using qRT-PCR. Localisation of BCL-2 was further investigated with immunohistochemistry/histocore analysis.

Findings/comment: The data suggest reduced endometrial apoptosis following SPRM, UPA exposure, though results were not statistically conclusive. Caspase-3 and BAX (pro-apoptotic markers) expression decreased following UPA treatment while BCL-2 (anti-apoptotic marker) expression increased. This study was funded by the SRF vacation scholarship 2018 and the EME (NIHR/MRC) programme: (12/206/52).

P142 Does obesity influence the human endometrial hypoxic response at menstruation to determine menstrual blood loss (MBL)?

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Background: Heavy menstrual bleeding (HMB) is common and debilitating but causes remain unclear. Hypoxia is thought to be present in the menstrual endometrium and drives post-menstrual repair to limit MBL. The contribution of obesity to MBL remains undetermined. We hypothesise:

1. Obesity increases MBL
2. This is due to an impaired hypoxic response at menstruation.

Aims:

1. To determine if increased body mass index (BMI) is associated with increased MBL.
2. To compare the hypoxic marker carbonic anhydrase IX (CAIX) and the expression of hypoxia regulated repair factors in non-obese and obese women with HMB and normal menstrual bleeding (NMB).

Methods: With ethical approval and consent, 121 women completed a pictorial bleeding assessment chart (PBAC) and BMI measurement. To assess endometrial markers of hypoxia, late secretory/menstrual endometrial samples were collected from a subset of women (n=20) and their MBL objectively measured (alkaline haematin method: HMB > 80ml). Women with fibroids >3cm or endometriosis were excluded. CAIX was analysed by immunohistochemistry and hypoxia-regulated repair factors (ADM, VEGFA, CXCR4) quantified by qRT-PCR.

Results: There was a significant correlation between BMI and PBAC score (R squared 0.052, p=0.012). CAIX immunostaining localized to the glandular epithelium and revealed no differences between non-obese and obese (BMI > 30) women regardless of MBL. There were no differences in mRNA levels of ADM, VEGFA or CXCR4 at menstruation between non-obese and obese women. A non-significant trend of decreased mRNA levels was seen in non-obese women with HMB compared to NMB.

Conclusions: There is a positive correlation between women's BMI and their MBL assessment. However, we found no evidence of impaired endometrial hypoxia at menstruation in women with a high BMI. Differences in other factors known to influence MBL (e.g. inflammatory markers, coagulation factors) remain to be determined in endometrium from women with and without obesity.

P143 Validation of Endometriosis Fertility Index (EFI) for non-ART pregnancy in women with endometriosis associated infertility

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Objectives: To validate and predict the ability of EFI on time to non-ART pregnancy in women with endometriosis associated infertility.

Methods: In a prospective cohort study, 72 infertile women with endometriosis undergoing laparoscopic surgery were assessed by r-ASRM and EFI scoring done post-surgically using historical and surgical factors. Patients were followed by ovarian stimulation and IUI for the subsequent one year. Primary outcome was clinical and ongoing pregnancy rate, and time taken to achieve pregnancy.

Results: The cumulative pregnancy rate was 33.3% and was significantly higher with EFI scores > 7 ($p=0.003$). The sensitivity and specificity of EFI in predicting clinical pregnancy was 95.8% (95% CI; 79.8-99.3) and 56.2% (95% CI; 37.9-66.3) respectively. The AUC was 0.90 (95% CI; 0.82-0.97). With r-ASRM the sensitivity and specificity in predicting clinical pregnancy was 83.3% (95% CI; 64.2-93.2) 79.2% (95% CI; 65.7-88.3) respectively. The AUC was 0.92 (95% CI; 0.86-0.98). For predicting ongoing pregnancy, the sensitivity and specificity of EFI was 94.7% (95% CI; 75.4-99.1) and 50.9% (95% CI; 37.9-63.9) respectively with AUC being 0.86 (95% CI; 0.76-0.96). Predicting ongoing pregnancy with r-ASRM had sensitivity and specificity of 84.2% (95% CI; 62.3-94.5) and 73.6% (95% CI; 60.4-83.6) respectively. The AUC was 0.87 (95% CI; 0.79-0.96). The odds of clinical pregnancy with EFI score > 7 was 29.6 as compared to 19.0 with r-ASRM score < 21 ($p=0.001$). The odds of ongoing pregnancy was 18.7 with EFI and 14.8 with ASRM score ($p=0.006$). The probability to conceive steadily increased from 0.05 to 0.3 between second and fifth month and plateaued at sixth month after surgery.

Conclusions: This study validates the EFI scoring and suggests its ability to predict time to non-ART pregnancy in women with endometriosis associated infertility.

P144 A computational method to assess the endometrial response to seminal plasma

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Introduction: The immunomodulatory role of seminal plasma in the establishment of pregnancy and subsequent maternal foetal allograft tolerance is well recognised. However, the quantitative evaluation of endometrial immune effector cell recruitment in this process is unclear. This study aimed to use an open-source digital pathology platform to characterise the density and location of immune infiltrates in murine post-coital female reproductive tracts.

Methods: Oestrous female CD1 mice were mated with vasectomised males and culled 6, 24 and 96h post-coitum ($n=3$). Females at oestrus, metoestrus and dioestrus were control groups. Uteri were immunohistochemically stained for CD3/FoxP3 (T-cells/regulatory T-cells), CCR3 (eosinophils), F4/80 (macrophages), NCR1 (natural-killer cells), CD19 (B-cells), and CD207 (dendritic cells). A novel automated workflow was developed with the open-source platform QuPath. Using superpixels and a machine learning classifier, endometrium and myometrium were delineated into discrete compartments. Tissue folds/over-stained regions were excluded, reducing false positives, and cells were automatically counted. Subsequent data were analysed using Kruskal-Wallis with Dunn-Bonferroni post-hoc tests.

Results: Macrophages were the predominant cell type in all groups. Within the endometrium, macrophage infiltration peaked in the vasectomised male-mated group at 24h post-coitum, accounting for 33.3% total cells compared to 8.0% cells at baseline (oestrus) ($p=0.020$), falling to 12.8% by 96h. This pattern was mirrored in the myometrium, peaking at 19.8% total cells at 24h. Control group macrophage density remained stable at under 10.0% total cells. All other immune effector cells remained at low densities (0.01-2% total cells) regardless of time point or treatment.

Discussion: This study demonstrates that immune effector cell infiltrates can be accurately quantified using advanced digital pathology. This approach reduces resource requirements and time commitment while increasing measurement accuracy. These findings also indicate that macrophages are the predominant immune effector cells in the response to seminal plasma and that their transient infiltration peaks at 24h post-coitum.

P145 The challenge of endometriosis diagnosis and treatment

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Aims and objectives: What can explain delays in diagnosis? To What can be improved for the management. Do healthcare professionals recognise the symptomatology?

Background: One in ten British women have endometriosis^[1], or which mean time to diagnosis exceeds 7 years^[1]. The perspective of health care professionals about this delay, opinions on current guidelines, and problems in training is poorly understood. Studies have not considered misdiagnosis of endometriosis from lack of symptom awareness.

Design: Qualitative semi-structured interviews ($n=8$) and quantitative study ($n=6$) of health care professionals.

Methods: We sought out health care professionals having in-depth knowledge of endometriosis;

- 3 gynaecologists & 1 endometriosis specialist nurse from local Endometriosis Centres^[2];
- 3 GPs with experience of patients with endometriosis
- 1 pain management specialist with special interest in pelvic pain.

Data were collected via face to face interviews, recorded, transcribed and analysed for recurrent themes. For quantitative study, we asked participants how likely they would diagnose endometriosis in 7 cases presented to them using a questionnaire, using a Likert scale of 1-5.

Results: Qualitative research identified 4 recurrent themes: diagnosis delays, awareness in primary care, management difficulties and NICE guidelines. In 4/7 cases, correct diagnoses were made by the participants. For 3/7 the participants misdiagnosed the case. The misdiagnosed cases presented patients GI or urinary tract endometriosis and early stages of endometriosis without any visible lesions.

Conclusion: Of course, a bigger sample size would be more informative. The cases presented were limiting, with information was provided beyond the presenting symptoms. Qualitative interviews provided vital information on the management and diagnosis of endometriosis and recommendations for future practice. The quantitative research showed how the symptomatic overlap of endometriosis can pose a challenge for health care professionals. Further education at the primary care level and more referrals to Specialist Centres will improve patient outcomes.

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P146 Tubal patency testing in low risk patients presenting with infertility

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Introduction: Tubal factor infertility affect 11 to 30% of couples presenting with infertility^[1]. Risk factors for tubal disease include previous Chlamydia infection/pelvic inflammatory disease (PID) and previous pelvic surgery. In our hospital, tubal patency is most often investigated through hysterosalpingogram (HSG).

Objective: Our study aims to identify the incidence of tubal disease in patients with no history of PID or pelvic surgery and in patients who has previous had confirmed intrauterine pregnancy.

Method: This was a retrospective study of all patients who had HSG between June 2016 and May 2017 (1 year) in our hospital. Their clinical history was obtained from their hospital notes. 117 patients were identified by the radiology department and included in our study.

Results: Of the 117 patients included in the study, 19 (16%) patients reported previous Chlamydia/PID while 27(23%) patients had previous abdominal/pelvic surgery. On HSG, 95(81%) patients had bilateral free spill from tubes confirming tubal patency, 15 (13%) had unilateral spill while 7 (6%) had no free spill of contrast. Of the 15 patients with no risk factors for tubal disease and with previous confirmed spontaneous intrauterine pregnancy (including termination of pregnancy and miscarriage diagnosed on ultrasound scan), 14 (93%) patients had bilateral patent tubes while it was inconclusive for the remaining 1 patient due to excessive spill of contrast from the contralateral tube. In comparison, bilateral free spill was demonstrated in 91% patients with no risk factors (regardless of previous intrauterine pregnancy), 69% patients with previous Chlamydia/PID and 48% patients with previous abdominal/pelvic surgery.

Conclusion: In the subset of patients with no risk factors for tubal disease and particularly in those with previous intrauterine pregnancy, it is reasonable not to investigate for tubal patency given the low risk of demonstrable tubal disease balanced with the risk/complication of HSG including pelvic infection and patient tolerability of HSG.

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P147 The effect of Intralipids and Vitamin D on gene expression of potassium channels in human infertile secretory endometrium

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Introduction: Infertility affects approximately 1 in 7 couples in the UK with implantation failure accounting for a significant number of reduced IVF and natural pregnancy rates. Endometrial receptivity is key to successful implantation and understanding its molecular determinants may hold important clues for implantation defects. Endometrial cells are exposed to a constantly changing microenvironment factors typically pH, lipids, oxygen tension and electrolytes (Ng et al., 2017) including a 6-fold higher potassium (K⁺) concentration than is found in plasma (Patel et al., 2013). Until now there is no

known direct relation between Intralipids and vitamin D and their effect on embryo implantation despite being used clinically worldwide (Lerchbaum and Obermayer-Pietsch, 2012).

Study design: This is a lab based study investigating effect the Intralipids and the Vitamin D on potassium channels in human infertile secretory endometrium (n=9) who experienced ≥ 1 implantation failure. In each group we compared the gene expression of KCNK9 (TASK-3), KCNK10 (TREK-2), KCNK17 (TALK-2) and KCNMA1 (BKCa) under both Intralipids (12.5ml/kg) and Vitamin D (2.5mg/kg). The experiment was carried out using qPCR and flow cytometry.

Results: Expression of all potassium channels (KCNK9, KCNK10, KCNK17 & KCNMA1) and housekeeping genes (ACTB, YWHAZ & TOP1) in all samples were tested. After treating the endometrial samples with Intralipids and Vitamin D, one of the channels (KCNK17) showed significant altered expression ($P < 0.05$) to both treatments. Translation to protein for the potassium channels was confirmed by flow cytometry.

Limitations: Larger (n) number of samples is needed. Criteria of target group is so precise not allowing a high recruitment flow. Heterogeneity of endometrial tissue makes it difficult to identify which cell types showed the expression. Implications of findings: Will provide new knowledge on the role of potassium channels contributing to normal and infertile endometrial function and identifying novel targets for fertility regulation.

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Endocrinology

P148 Re-organisation of FSHR di/oligomeric complexes by FSH glycosylation variants positively modulates FSHR-dependent signal activation

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The actions of follicle stimulating hormone (FSH) and its associated G protein-coupled receptor (FSHR) are essential for reproduction. They are primary targets for assisted reproductive techniques such as IVF, therefore, interest in understanding what controls their functions in reproductive health and disease remains high. FSH is a heterodimeric glycoprotein hormone, and recent studies have identified two predominant glycosylation variants of FSH, with differing bioactivities, with hypoglycosylated FSH (FSH21) more bioactive than hyperglycosylated FSH (FSH24).

Our previous studies have shown that an important mode of regulating gonadotrophin receptor function is via formation of dimers and oligomers, therefore our study aimed to determine the effect of differential FSH glycosylation on FSHR di/oligomer formation.

Using HEK293 cells transiently expressing HA-FSHR, we first confirmed that in our cell model FSH21 more potently activated CREB and ERK phosphorylation in comparison to FSH24. Using our recently developed super-resolution imaging technique of photoactivated dye localisation microscopy (PD-PALM), we next imaged and quantified the effect of FSH21 and FSH24 on the cell surface landscape of FSHR monomer, dimers and oligomers, to a resolution of $< 10\text{nm}$. Acute 2-minute treatment with 30ng/ml FSH21 significantly decreased the number of FSHR homomers observed in comparison to basal control, and importantly, in comparison to FSH24.

Analysis of the types of associated FSHR complexes formed revealed that FSH21 enriched FSHR dimers, suggesting that dissociation of FSHR oligomers into dimers and monomers enhanced FSH21-dependent signal activation. Interestingly, FSH24 had no effect on neither the total number of associated FSHR, nor the types of complexes formed. More chronic 5- and 15-minute stimulations showed FSH21-dependent FSHR reorganisation, with comparable total number of associated FSHR and types of complexes formed to basal and FSH24. These data suggest that reorganisation of FSHR complexes by differential FSH glycosylation can fine-tune signal strength and duration, with potential impact on physiological responses.

P149 Prevalence of POSEIDON strata of poor prognosis in patients undertaking their first IVF cycle

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Background: The POSEIDON group (Patient-Oriented Strategies Encompassing Individualized Oocyte Number) proposed a new stratification of assisted reproductive technology (ART) in patients with a reduced ovarian reserve or unexpected inappropriate ovarian response to exogenous gonadotropins^[1]. The objective of this study was to evaluate the prevalence of the POSEIDON strata in the patients undertaking their first IVF cycle.

Methods: Prospective cohort study of 400 patients (3600 biological samples) undertaking their first IVF cycle, with pre-treatment data on anti-müllerian hormone (AMH) and antral follicle count (AFC) to facilitate classification into POSEIDON Groups 3 and 4 and post-stimulation data to classify those with an unexpected poor or suboptimal response into POSEIDON Groups 1 and 2, and their respective subclasses of a and b if fewer than four oocytes; or four to nine oocytes were retrieved after standard ovarian stimulation.

Results: Participants have a mean age of 33.8 ± 7.4 years with a median (IQR) AMH of 15.8 pmol/l (8.8, 28) and AFC of 12 (8, 17). 68% of all cycles met the criteria for a POSEIDON group. Pre-treatment the prevalence of POSEIDON group 3 was 8.25% and POSEIDON group 4 was 18.0%. The mean number of oocytes retrieved was 9.1 ± 5.2 for the whole cohort. The prevalence and mean oocyte yield for each of the POSEIDON groups post-treatment was group 1a 4.5% (0.67 oocytes), 19.5% group 1b (6.9 oocytes), 3% group 2a (1.4 oocytes) and 14.75% group 2b (6.8 oocytes). Only 128 of the 400 patients (32%) did not meet the POSEIDON criteria prior to or after treatment and had a mean yield of 14.2 ± 4.9 oocytes.

Conclusion: The POSEIDON strata encompass a large proportion of patients undertaking IVF. The high prevalence of the suboptimal ovarian response POSEIDON strata (1b and 2b) suggest clarity over "standard ovarian stimulation" may be useful.

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P150 RNA-Sequencing of PCOS granulosa-lutein cells reveals differential regulation of genes implicated in cholesterol biosynthesis and steroidogenesis

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Polycystic ovary syndrome (PCOS) is a common endocrinopathy that is associated with anovulatory infertility and menstrual irregularities. Although its aetiology and pathophysiology remain unclear, granulosa cell dysfunction is a notable feature of the syndrome. In this study, we carried out a genome-wide comparison of gene expression in granulosa-lutein cells from women with PCOS. Granulosa-lutein cells were retrieved from women undergoing in-vitro fertilisation with either normal ovaries and regular cycles, polycystic ovaries and irregular cycles (anovPCOS) or polycystic ovaries with regular, ovulatory cycles (ovPCO).

RT-qPCR and RNA-Sequencing together with bioinformatic analysis were used to identify genes in granulosa cells that may be implicated in the pathogenesis of PCOS. RNA-Sequencing identified 21175 genes in granulosa-lutein cells with women with PCOS showing a distinct global transcriptional profile of 450 differentially expressed genes. Gene Ontology, pathway and network analyses highlighted a substantial reduction in over 20 cholesterol biosynthesis and metabolism genes in women with PCOS. Several members of the steroidogenic gene network were also affected including a reduction in CYP11A1 and HSD17B1 and an increase in SULT1E1. Finally, granulosa-lutein cells were cultured in the presence or absence of androgen, and the results showed that dysregulation of a subset of cholesterol metabolism genes and SULT1E1 expression in women with PCOS may be, at least in part, a function of androgen action.

Granulosa cells play a major role in ovarian steroid synthesis including estrogen and progesterone. The significance of altered expression of genes involved steroid and cholesterol metabolism remains to be determined but support the hypothesis of a compensatory mechanism. These results lend further support to the notion of aberrant metabolic and endocrine function in granulosa cells of women with PCOS.

P151 Biological and non-biological parenthood in Turner Syndrome (TS): A 17-year single-centre cohort study

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Purpose: Infertility and the inability to become a biological mother are major concerns for women with TS. We set out to provide data on the routes to parenthood taken by a cohort of women with TS.

Methods: Single-centre cohort study of adult women with TS over a 17-year period.

Results: 124 women with TS attended between 2000 and 2017. Median age: 33 years (range: 18-74). Karyotypes: 45XO n=43 (30.6%); 45XO,46XX n=22 (17.7%); mosaicism with X-ring n=12 (9.7%); mosaicism with Y-chromosome n=7 (5.6%); Isochromosome-X n=6 (4.8%); 45XO,47XXX n=5 (4%); 45XO,46XX,47XXX n=2 (1.6%); Other X anomalies n=27 (21.7%). Of the 124 women, 8 (6.5%) achieved 22 spontaneous conceptions resulting in 13 singleton live-births and 9 miscarriages. 12 women achieved 14 live-births (2*twin pregnancies) with ART using oocyte donation (OD); 3 pregnancies were complicated by: placenta increta, hypertension and pre-eclampsia, respectively. 2 women adopted and one was acting as a step-parent. 101 women were not known to be in a parent role. Of these: 3 were on OD waiting lists; 9 were considering OD; 2 were

ineligible for NHS-funded OD; one underwent oocyte cryopreservation (not yet used). Five had bilateral salpingo-oophorectomies as children due to the presence of Y chromosome. Two had MRKH syndrome in addition to TS, of whom one had biochemically intact ovarian function. 96 women had primary amenorrhoea. 28 (23%) women had spontaneous menarche and regular periods. Their karyotypes were: 45XO,46XX (n=11); mosaicism with X-ring (n=3); 45XO,47XXX (n=3); Isochromosome-X (n=2); 45XO,46XX,47XXX (n=2); 45XO (n=1); Other X anomalies (n=6). Of whom 16 developed secondary amenorrhoea at a median age of 32 years (range: 18-55).

Conclusion: Various routes to parenthood are open to women with TS, including biological parenthood. Early identification of patients who might achieve biological parenthood, and referral to fertility specialists for reproductive counselling and fertility preservation assessment is advisable.

P152 The role of androgen signalling in uterine morphogenesis and cancer in a mouse model of PCOS

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Background: Uterine cancer is one of the leading malignancies among women worldwide. Increased risk of developing uterine cancer has been associated with a number of contributing factors including age, obesity, unopposed oestrogen, hormone replacement therapy and polycystic ovary syndrome (PCOS). Although pathophysiology of PCOS has not been fully understood, its main biochemical feature is excessive ovarian androgen production. We hypothesised that excess androgen and high fat diet drive re-programming of certain genes in-utero resulting in manifestation of the endocrine, metabolic and long-term health problems later in adult life and possibly contributing to the risk of uterine cancer. In this study we investigated the role of prenatal exposure to excess androgen and high fat diet in development of uterine cancer in mouse model of PCOS.

Methods: Expression of genes serving as cancer markers, signalling molecules, cell cycle regulators and genes thought to be implicated in normal and disordered uterine function, was examined by real-time qRT-PCR. Detailed immunohistochemistry was performed for quantitative analysis of protein expression (Ki67 - proliferation marker and Foxa2 - uterine gland marker) and morphological changes in murine uteri.

Results: Endometrial and total uterine areas were greater in animals on high fat diet regime. Animals treated with 5 α -dihydrotestosterone (DHT) showed decreased proliferation of glandular epithelium of the uterus but qRT-PCR analysis revealed significant upregulation of the stromal-mesenchymal regulator *Igf1*. We also observed that high fat diet and DHT have additive effect on upregulating *Foxa2*, a key molecule involved in adenogenesis.

Conclusion: Taken together, our findings do not unambiguously elucidate the significance of prenatal excess androgen and high fat diet on uterine cancer pathogenesis. Nevertheless, they clearly suggest that prenatal exposure to excess androgen and high fat diet can affect expression of genes involved in uterine morphogenesis, resulting in disrupted uterine and uterine gland physiology.

P154 Acetic acid and Arachidonic acid profile of bovine milk as oestrus detection method during spontaneous oestrus in lactating cows

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Oestrus is a behavioural strategy to ensure that the cow is mated close to the time of ovulation. This is especially in dairy herds using artificial insemination. However, one of the predominant reproductive dysfunctions causing sub-fertility in dairy cows is silent oestrus. Milk is a readily available medium with potential for oestrus detection. However, there are no known published studies relating the level of fatty acids to oestrus activity of spontaneously cycling cows. Therefore, the present study is designed to determine milk fatty acid profile of dairy cows during oestrus and di-oestrus and to investigate whether these could be used as an additional method of oestrus detection.

Lactating Holstein Friesian cows (n=32) were used. Cows were fed a TMR ad libitum. Oestrus was detected using milk samples on the day of oestrus and on day 14 of the subsequent cycle and analysed for fatty acids concentration using gas chromatography (Hewlett-Packard - 7820A, Agilent Technologies Inc. Germany). The data were analyzed by one-way factorial ANOVA (GenStat 17th edition). From 32 oestruses, the concentration of acetic acid (C2:0) in milk were higher (P=0.003) on the day of oestrus (297.0 $\pm$ 18.5mg/100 ml) compared to the day of di-oestrus (229 $\pm$ 11.9 mg/100ml). However, on the day of di-oestrus (day14) arachidonic acid (C20:4n6c) concentrations in milk samples were higher (P=0.003) in comparison to the day of oestrus (0.60 $\pm$ 0.03 g/100g of FA vs 0.41 $\pm$ 0.05 g/100g of FA), respectively.

In conclusion, as far as we are aware this is the first study to show an increase in milk concentration of acetic acid during the oestrus period and the concentration of arachidonic acid during di-oestrus period. The finding of this study suggests that it may be possible to use concentration of certain fatty acids in milk to determine the time of oestrus in dairy cows.

Oocytes

P155 The effects of PADI6 siRNAi knockdown in bovine GV oocytes on gene expression and preimplantation embryo development

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PADI6 is a maternal effect gene that is critical for oocyte competence and for the maternal-zygotic transition (MZT). Together with the SCMC, it is involved in transcription, epigenetic regulation and cytoskeletal organisation. PADI6 is essential for the maintenance of oocyte cytoplasmic lattices that function as cytoskeletal machinery and storage components for maternal factors. Previously, we mapped the expression of PADI6 and other MEGs across bovine preimplantation embryo development. Furthermore, PADI6 gene knockdown via dsRNA microinjection into GV oocytes (~74% PADI6 knockdown (n=20) versus controls (n=21)) did not affect oocyte viability or maturation, but significantly altered the expression of four epigenetic regulators, DNMT3A, DPPA3, TRIM28 and ZFP57. Notably, DNMT3A, TRIM28 and ZFP57 directly interact at specific loci to maintain imprinted genes.

The current study aimed to i)map the expression of PADI6 targets DNMT3A, DPPA3, TRIM28 and ZFP57 across bovine preimplantation development; ii)use RNA sequencing to further analyse the transcriptome of PADI6 knockdown oocytes; iii)investigate the role of PADI6 in preimplantation development by gene knockdown in GV oocytes followed by IVF. DPPA3 was highly expressed before MZT and was significantly decreased in morula to blastocysts ($p<0.05$). Conversely, DNMT3A and ZFP57 had little to no expression before MZT but later expression increased ($p<0.05$). TRIM28 expression was constant throughout. RNA sequencing identified genes CRSP3, ELOVL6 and POSTN that were significantly altered in PADI6 knockdown MII oocytes with involvement in transcription, lipid metabolism and extracellular matrix restructuring respectively. Finally, preliminary data from embryos derived from PADI6 knockdown MII oocytes suggest an increase in embryonic arrest at 4-8 cell stage compared to control embryos.

Transcriptome analysis following RNA sequencing will further evaluate changes in epigenetic regulators, oocyte quality markers and key developmental pathways. This study highlights downstream effectors of PADI6 and suggests a role for PADI6 in early embryo development and MZT.

P156 Effect oocyte factors on granulosa cells differentiation and cumulus- extracellular matrix organization

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Previously, we have demonstrated the expression of growth differentiation factor 9 (GDF9) in porcine oocytes, cumulus cells (CC) and mural granulosa cells (GC; Prochazka et al. 2004). Recently, we have shown that bone morphogenetic factor 15 (BMP15), another oocyte produced factor, affected differentiation of GC and organization of cumulus extracellular matrix (ECM) in porcine ovarian follicle.

We detected a significant increase in the expression of AREG and TNFAIP6 (both at 16 h) and CYP11A1 (at 24 h) in FSH/LH-stimulated oocyte-cumulus complexes (OCC) due to the action of BMP15 compared to complexes cultured only with FSH/LH (Nagyova et al. 2017). The third investigated oocyte-produced factor was fibroblast growth factor 10 (FGF10), since it has been shown that FGF receptors utilized by FGF10 are expressed in bovine CC and oocytes, and that FGF10 affects bovine oocyte maturation in vitro.

We used porcine GC or OCC in vitro stimulated with gonadotropins to evaluate principal maturation processes such as resumption of meiosis, cumulus expansion, hyaluronan (HA) synthesis and steroidogenesis in absence/presence of FGF10. In contrast to serum-free conditions, the FSH/LH/FGF10- induced cumulus expansion associated with significant increase in HA synthesis and its retention within expanded cumulus ECM was observed when medium was supplemented with serum. Concerning differentiation, both, basal and gonadotropin-stimulated progesterone (P4) measured after culture of GC as primary monolayer in the presence of FGF10 was not changed in comparison to control. Moreover, FGF10 had no effect on P4 production by porcine gonadotropin-stimulated OCC in serum-supplemented medium. However, FGF10 increased P4 production by OCC cultured in serum-free conditions. Interestingly, FGF10 (at 10ng/ml) significantly increased androstenedione-stimulated E2 production by porcine GC.

References:

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P157 Prolonging prometaphase in meiosis I mouse oocytes to prevent aneuploidy

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Successful mitosis requires that cyclin B1:CDK1 kinase activity remains high until all chromosomes are correctly aligned on the mitotic spindle. It has therefore been unclear why, in mammalian oocyte meiosis, cyclin B1 destruction begins before chromosome alignment is complete. This feature of oocyte biology has previously been linked to the high frequency of division errors in human oocytes; the number one genetic cause of miscarriage. However, in mouse oocytes we find that though cyclin B1 destruction begins ahead of chromosome alignment, CDK1 activity remains high due to an excess of cyclin B1; mouse oocytes then rarely divide with errors.

Here we investigated how free non-CDK1-bound cyclin B1 is targeted for destruction ahead of CDK1-bound cyclin B1 to preserve essential cyclin B1:CDK1 activity. By microinjecting fluorescent free and CDK1-bound cyclin B1 reporter constructs we have demonstrated in live maturing mouse oocytes that free cyclin B1 is targeted for destruction up to 90 minutes ahead of CDK1-bound cyclin B1. Furthermore, we find that when we knock down the Cdc20 component of the degradation complex responsible for cyclin B1 destruction, only free cyclin B1 is targeted. Similarly, if we prevent chromosomes from completing alignment with the use nocodazole to destabilise microtubule attachments, only free cyclin B1 is targeted.

This demonstrates that the degradation machinery has a clear preference for free cyclin B1 earlier in the cell cycle and explains how cyclin B1:CDK1 activity is preserved. We suggest that an excess of free cyclin B1 acts as bait to divert the degradation machinery away from cyclin B1:CDK1 activity, preserving vital activity.

This finding represents an important step forward in understanding cell cycle regulation in mouse oocytes. It will now be of upmost interest to determine if this same mechanism operates in humans, both in competent oocytes and in women suffering infertility.

P158 Obesity induced changes in oocytes and the developmental potential of preimplantation stage embryos

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Background: Obesity is a major health issue and associated with various pathologies including infertility. Excessive adipose tissue accumulation can cause perturbation in regulation of hypothalamo-pituitary-gonadal axis and can have adverse effects on follicular microenvironment. The present study was aimed at correlating the cytoplasmic changes in the oocytes with developmental potential of embryos using murine model.

Objective: To understand the effect of diet induced obesity on lipid accumulation, oxidative and ER stress in oocytes.

Methods: Female Swiss albino mice (3 weeks) were fed with normal diet (control, N=12) or high fat diet (HFD, N=12) for 8 weeks after which mice were assessed for estrus cycling by vaginal cytology and biochemical analysis. The germinal vesicle (GV) stage oocytes (N=250) were subjected to in vitro maturation (IVM) and intracellular ROS (DCHFDA staining), lipid accumulation (Nile red staining), XBP1 expression (immunofluorescence) were assessed in oocytes. To assess the developmental potential, in vitro fertilization was performed and embryos were cultured till blastocyst stage. The fertilization, blastocyst rate and DNA integrity in blastocyst (TUNEL assay) were assessed.

Results: The GV oocytes collected from HFD fed female mice exhibited poor in vitro maturation potential ($p < 0.01$) and high lipid accumulation ($p < 0.05$). Increased intracellular ROS level ($p < 0.01$) and moderate increase in XBP1 expression indicated that oocytes from HFD group have high intracellular oxidative and ER stress. Further, fertilization ($p < 0.001$) and blastocyst rate ($p < 0.0001$) was significantly lower while, DNA damage in blastocysts was significantly higher ($p < 0.001$) when compared to control.

Conclusion: The diet induced obesity results in subtle cytoplasmic changes in oocytes which might contribute to poor developmental potential of embryos.

P159 The effect of a six week dietary intervention on the fatty acid composition of human follicular fluid

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Aims/objectives: The aim of this study was to investigate whether a six week dietary intervention, containing omega-3 fatty acids and vitamin D, taken by women prior to IVF or IVF-ICSI treatment altered the fatty acid composition of follicular fluid.

Content of presentation: One hundred and eleven women were randomized in a double blind manner to receive either the trial drink, containing 2 g of DHA and EPA and 10 micrograms of vitamin D, olive oil and olive oil spread or a placebo drink and sunflower oil equivalents, to consume daily for six weeks prior to oocyte retrieval. Follicular fluid was collected at oocyte retrieval for analysis of the fatty acids composition. Data was recorded on oocyte number, maturation and subsequent blastocyst formation.

Relevance/impact: A number of retrospective studies have suggested links between preconception diet and ART outcome but data from blinded randomised controlled trials is scarce.

Outcomes: Women randomised to the intervention group demonstrated a significant decrease in the percentage of linoleic acid ($p < 0.001$), and increases in the proportions of EPA ($p < 0.001$) and DHA ($p < 0.001$) in their follicular fluid. Palmitic acid was found in the highest levels ($28.50\% \pm 1.33$), followed by linoleic acid ($23.12\% \pm 2.86$), oleic acid ($17.36\% \pm 1.93$) and stearic acid ($12.01\% \pm 0.91$). No differences were observed in the number of eggs collected ($p = 0.540$) or the percentage of these that were mature ($p = 0.810$) or in the number of resulting blastocysts ($p = 0.356$).

Discussion: This study demonstrated that a targeted six week dietary intervention altered the fatty acid composition of follicular fluid. While no difference was seen in blastocyst formation rates after this intervention, diet is clearly shown to alter the environment of the maturing oocyte.

P160 Influence of follicle number on normal fertilisation and oocyte necrosis rates after ICSI

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The outcome of intracytoplasmic sperm injection (ICSI) is partially dependant on the oocyte's ability to withstand denudation and microinjection. Several studies have indicated that the oocyte's response to microinjection may be influenced by supraphysiologic estradiol levels, occurring as a result of follicle maturation during controlled ovarian stimulation (COS). The aim of this study was to determine if follicle number, assessed at the time of oocyte recovery set up scan, was associated with normal fertilisation and oocyte necrosis rates after ICSI.

Fresh ICSI cycle fertilisation and oocyte viability data was collected retrospectively from January 2016 to May 2018. Cycles in which COS was achieved by either antagonist or long down regulation regimens were included. Oocyte donation and surrogacy cycles were excluded from analysis. Cycles in which oocytes were split between conventional in-vitro fertilisation (IVF) and ICSI were also excluded. Fertilisation and Oocyte necrosis rates were categorised into one of three groups according to follicle number, group A: 1-5 follicles, group B: 6-10 follicles and group C > 10 follicles.

Normal fertilisation and oocyte necrosis rates were used as outcome measures. Normal fertilisation rate did not differ between groups A (66.9%), B (67.3%) and C (66.9%). Oocyte necrosis rate was lower in group A (2.7%) compared to groups B (7.4%) and C (7.7%). The findings of this retrospective audit suggest that although follicle number does not appear to influence the chance of normal fertilisation after ICSI it may be associated with oocyte damage leading to necrosis.

P161 Analysis of the transcriptome of individual GV and MII oocytes derived from prepubertal and postpubertal sheep

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Oocyte developmental competence requires synchronized nuclear and cytoplasmic maturation. Cytoplasmic factors including mRNA storage during the later stages of follicle growth and differentiation are essential for fertilization and embryogenesis before embryonic genome activation. Oocytes from prepubertal animals have compromised developmental competence, however, the molecular mechanisms are unclear.

The transcriptome of individual, denuded, GV and MII oocytes derived from postpubertal (GV_{po} and MII_{po}) and prepubertal (GV_{pp} and MII_{pp}) sheep were analyzed by strand specific total RNA Sequencing. 10 individual oocytes were analyzed per age group. Data quality was rigorously assessed during bioinformatic analysis which included: use of Scploid for aneuploidy detection; edgeR for differential expression and ontological analyses; and the R package mixOmics for heatmap plotting.

Many genes were downregulated (GV_{po} vs MII_{po}: 484; GV_{pp} vs MII_{pp}: 506 LogFC > 1) whereas comparatively few genes were upregulated (GV_{po} vs MII_{po}: 35; GV_{pp} vs MII_{pp}: 78 LogFC < -1). Irrespective of age, genes related to mitochondrial function and cellular antioxidant status were downregulated from GV to MII, whereas genes regulating cell cycle, chromosome condensation and separation were upregulated, collectively reflecting the energy requirements and biological changes associated with meiotic progression. Genes regulating integrin binding, glutathione GPX activity, serine-type endopeptidase pathways as well as endonuclease activity, positive regulation of inflammatory response and sorting endosome pathways were upregulated in GV_{po} and GV_{pp} respectively. Genes regulating hormone activity, protein maturation by iron-sulphur cluster transfer and PI3K signal pathways and genes involved in methylosome, centromere complex assembly and regulation of cellular response to TGF stimulus pathways were enriched in MII_{po} and MII_{pp} oocytes respectively.

GPX activity, PI3k signal and TGF stimulus pathways have been demonstrated to play critical roles during oocyte maturation. Further investigation is needed to identify how these pathways regulate oocyte developmental competence in pre-pubertal and post-pubertal sheep in vivo and in vitro.

P162 There is significant unexplained inter-cycle variation in ovarian performance during IVF treatment

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Introduction: The aim of this study was to investigate the extent of inter-cycle variation in oocyte yield between successive treatments, which cannot be explained by modifications in the drug regime.

Materials: Data from consecutive patients who had undergone at least two oocyte retrievals within the same year were retrospectively analysed. Poor responders were excluded. We measured the frequency of 2 outcomes that support the presence of unexplained inter-cycle variability:

- i) Unexpected ovarian response, which includes the scenarios: higher stimulation dose during the 2nd treatment did not lead to increased oocyte yield, lower stimulation dose during the 2nd treatment did not lead to reduced yield, same stimulation dose during the 2nd treatment led to increased or reduced oocyte yield, and
- ii) Paradoxical ovarian response, which includes the scenarios: higher stimulation dose during the 2nd treatment led to reduced oocyte yield, lower stimulation dose during the 2nd treatment led to increased yield.

Multivariable analysis was performed for both outcomes, adjusting for the operator, changes in drug protocol, the woman's age and the number of oocytes (1st retrieval). Observed as well as adjusted frequencies were reported.

Results:

- Data from 2136 patients were included.
- Using a 30% cut-off for detecting a difference in the oocyte yield, approximately 55% of women demonstrated unexpected response (adjusted 56% 95%CI 51%-62%). Moreover, 8% of women (adjusted 9% 95%CI 6%-13%) exhibited paradoxical response between treatments.
- Using a 10% cut-off for detecting a difference in the oocyte yield, unexpected ovarian response was experienced by 65% of women (adjusted 67% 95%CI 61%-72%), while paradoxical response was seen in 15% of women (adjusted 17% 95%CI 13%-22%).

Conclusion: As this original study suggests, the ovaries exhibit substantial intrinsic inter-cycle variability with regard to their potential to recruit oocytes. This should be considered when managing expectations of couples who require repeated IVF treatments.

P163 Impact of cryopreservation on developmental competence in slow frozen and vitrified human metaphase II oocytes

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Background: Modifications to ART methods of cryopreservation have led to significant improvements in IVF treatment cycles and donor egg programmes. Current methods of cryopreservation offer a means of fertility preservation (oocyte freezing) for reproductive age women who require harmful chemotherapy treatment or surgery following cancer diagnosis. Recent HFEA report^[1,2] indicates an increase of 10 and 19% in egg freezing and thaw cycles with a live-birth rate of 19% per ET. Despite evidence from the Developmental Origin of Health and Disease³ (DoHAD) and concerns over safety of cryopreservation, the possible impact of the procedure on long-term health of the resulting offspring is yet to be fully evaluated in ART.

Objectives: To investigate the impact of cryopreservation on oocyte gene expression, known molecular markers of stress and developmental competence in slow-frozen and vitrified human metaphase II oocytes.

Methods: Eighty-one matured and failed-to-fertilise (IVF/ICSI) oocytes surplus to treatment were consented to HFEA research licence R0026. Oocytes were allocated to slow freezing (n=31), vitrification (n=29) and control group (n=21). Poly(A)PCR amplification and Quantitative Polymerase Chain Reaction (qPCR) of cDNA was used to investigate gene expression patterns.

Results: Similar post-thaw survival rates were obtained following slow freezing (67.7%) and closed method of vitrification (72.4%). Several oocytes expressed genes linked with stress response; developmental competence and DNA damage (ATR, ATM, BRCA1 and 2, RAD50, TP53, HMGB2, MGMT and OCT4) at low levels. OCT4 appears to be down-regulated in the vitrified group relative to control (p=0.0104) and slow freezing group (p=0.0003). However, no statistical significant difference was observed between slow and control group (p=0.998).

Conclusion: This study reveals a potential means of evaluating the impact of cryopreservation on oocyte developmental competence. Also result shows that both methods of cryopreservation differentially modify gene expression pattern. This modification may potentially perturb oocyte genomic integrity, fertilisation and subsequent embryo development.

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P164 In-vitro potential of follicles/oocytes/putative germline stem cells obtained from African and Asian elephant ovaries

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Background: Fertility in captive female elephants can be compromised resulting in premature sterility, thus impacting on species conservation. Wild elephants however can ovulate into the 7th decade of life. Our study investigated the developmental potential in-vitro of follicles and oocytes from elephant ovarian tissue and isolated cells expressing the germline marker DDX4 from young and mature adult elephant ovaries.

Method: Cortical tissue was obtained from two pairs of elephant ovaries following euthanasia; a wild African elephant (19y) and a captive Asian elephant (60y). Tissue fragments from each animal were assigned to 4 groups; 1)fixation for assessment of follicle distribution 2)culture to assess follicle activation in-vitro 3)needle-dissection of growing follicles to assess in-vitro oocyte development and 4)isolation of putative germline stem cells using fluorescence activated cell sorting (FACS).

Results: Healthy follicles were observed in all tissues. Early primary follicles constituted the majority of the 19-y-old's ovarian reserve. All in-vitro activated follicles were morphologically abnormal. Fifteen growing follicles were dissected in total (African n = 13; Asian n = 2); follicles from the African elephant ovary grew in culture over 10 d; oocyte complexes were released and incubated; cumulus expansion was observed. Two populations of cells (DDX4-positive and DDX4-negative) were sorted from dissociated tissue by FACS. In DDX4-positive cells, DDX4 protein expression was confirmed by fluorescent immunocytochemistry.

Conclusion: Wild and captive adult elephant ovaries showed evidence of ovarian activity. Growing follicles were isolated and in-vitro oocyte development achieved in a young adult elephant. The normality, maturation and fertilisation potential of in-vitro grown (IVG) elephant oocytes has yet to be determined. Cells expressing DDX4 can be isolated from both young and mature elephant ovaries. Further investigation is required to assess the developmental potential of these cells and their possible impact for sustaining captive or endangered elephant populations.

Embryo

P165 Blastocyst survival: vitrified in Cook media and warmed in Vitrolife media

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Objective: Does the use of a different company's media for vitrification and warming of blastocysts affect the survival rate. It has always been good practice to vitrify and warm in media manufactured by the same company since media composition is optimised to maintain high survival rates. However, circumstances, such as supply issues, can occur where it becomes necessary to warm blastocysts in a different media to the one used for vitrification. This paper reports a situation whereby blastocysts vitrified in Cook media were warmed in Vitrolife media.

Methods: A total of 94 blastocysts that had been vitrified in Cook media (Sydney IVF Blastocyst Vitrification Kit, K-SIBV-5000) on CMV Cryologic straws were warmed in Vitrolife warming media (Rapidwarm™ Blast, 10120) according to the manufacturers' protocols. 69 of these were embryos donated for training and 25 were from clinical cases. In comparison 488 blastocysts that had been vitrified in Cook were warmed in Cook media (Sydney IVF Blastocyst Warming Kit, K-SIBW-5000). The warmed blastocysts were placed in culture media for one hour and survival was assessed according to percentage of cells survived (>50%) and degree of re-expansion of the blastocyst seen after 1 hour (>30%). Fisher's exact test was used to calculate the p value.

Results: There was no significant difference in survival rate of blastocysts warmed in Vitrolife media vs Cook media (93.6% vs 97.3%, p-value 0.1).

Conclusion: This study demonstrates that the survival of blastocysts vitrified in Cook media but warmed in Vitrolife media was comparable to blastocysts vitrified and warmed in Cook media. Implantation, clinical pregnancy and live birth rates are awaited but will be calculated and available for Fertility 2019.

P166 Can an elective single embryo transfer policy be implemented in a clinic treating exclusively private patients?

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Introduction: A multiple pregnancy is the most significant obstetric complication following fertility treatment. In 2009 the HFEA required fertility clinics to develop a multiple birth minimisation strategy. NHS clinical commissioning groups impose a compulsory single embryo transfer policy for funded treatment. In contrast private clinics must implement and establish their own strategies.

Aim: Can a clinic treating exclusively private patients implement an eSET policy in eligible patients under 39 years old?

Method: A retrospective analysis of all patients under 39 during the clinic's first operational year. Data was obtained from the IDEAS database and excluded oocyte donation cycles. eSET is recommended for all fresh blastocyst transfers (Grade A or B, modified Nuture grading scheme) in patients under 39. Three embryos exhibiting 6-8 cells on day 3 with less than 20% fragmentation and regular blastomeres are required for blastocyst culture. If the criteria for blastocyst culture is not met two cleavage stage embryos are transferred.

Results: 181 fresh embryo transfers were performed in patients under 39. Overall multiple pregnancy rate of 11.7%. 85 eSET cycles, 82 blastocyst stage and 3 cleavage stage. 73 DET cycles, 52 cleavage stage and 21 blastocyst stage with a resulting multiple pregnancy rate of 26.3% and 45%. 23 non elective SET's, 14 cleavage stage and 9 blastocyst. Double blastocyst transfer did not significantly increase the clinical pregnancy rate 52.4% versus 63.3% ($P=0.45$) but was associated with a significantly higher multiple pregnancy rate 45% versus 1.9% ($P=0.0004$). 2.4% of patients fulfilling eSET criteria requested the transfer of two top quality blastocysts against clinical advice (multiple pregnancy rate 50%).

Conclusion: A private clinic can implement an effective eSET policy by committing to staff and patient education and achieve a multiple pregnancy rate consistent with HFEA guidelines.

P167 Do patients (<35 years) who have one embryo replaced at the blastocyst stage on weekends have better success rates than the same group of patients during weekdays?

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Aim: The purpose of this study was to examine whether weekend embryo transfers (ET) are likely to yield better clinical pregnancy rates than weekday ETs. This is based on the theory that patients are perhaps likely to be more relaxed, less anxious on weekends, than those patients undergoing the same procedure during the week.

Materials and methods: A total of 120 patients were included in this retrospective study from May 2016 - July 2018. Patients were categorised into two groups based on their demographics, cycle characteristics and ET day. The clinical pregnancy rate between both groups was compared.

Results: A total of 87 patients underwent ET's on weekdays ($n=58$) or weekends ($n=29$). Among these two groups, the weekday group had a 63.8% ET clinical pregnancy rates and the weekend group had a 58.6% clinical pregnancy rate.

Conclusion: There was no statistically significant difference in the success rates and outcome of embryo transfer between the two groups.

P168 Do subsequent IVF cycles increase success rates?

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Aim: The aim of this study was to determine whether subsequent IVF cycles increase the likelihood of the clinical pregnancy rate in women by age and treatment type. Specific objectives were to determine: whether there was an increase in the clinical pregnancy rate within each cycle and as to how these varied by age and treatment types.

Method: Retrospective analysis of 206 stimulated IVF/ICSI cycles in was performed from January 2016 to January 2018. Patients were stratified into three age groups: 30-35 years ($N=76$); 36-39 years ($N=63$); 40-44 years ($N=67$). Data was analysed according to patient age and treatment type.

Results: Across all age groups and treatment types, the clinical pregnancy rate for cycles 1, 2, 3 and 4 were 33.4%, 20.8%, 10.2%, and 1%. The clinical pregnancy rate for patients between 30-35-years old undergoing cycles 1, 2, 3 and 4 were 36.4%, 32.9, 28.5% and 0%. The clinical pregnancy rate for patients between 36-39 years-old undergoing cycles 1, 2, 3 and 4 were

34.2, 32.7%, 18% and 0%. The clinical pregnancy rate for patients between 40-44 years-old undergoing cycles 1, 2, 3 and 4 were 15.4%, 11%, and 7.3%.

Conclusion: There was a slight decline in the clinical pregnancy rate for patients undergoing subsequent IVF cycles. For patients attempting a third or fourth IVF cycle, the decline was considerably greater. However, this was expected due to a smaller cohort of patients in this group.

P169 An effective laboratory strategy that has reduced multiple pregnancy rates from 20% to 3.4%

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Rationale: Historically, the unit was not able to reduce the multiple pregnancy rate (MPR) below the HFEA target without compromising the clinical pregnancy rate (CPR). The IVF multiple births within the Hospital, amounted to £1.17 million in neonatal costs over 2 years. In response, the laboratory underwent investment to optimise our embryo culture and selection systems, equipment and staffing patterns. This strategy enabled the implementation of a strict mandatory single embryo transfer (mSET) policy which would not be detrimental to clinical pregnancy rates (CPR).

Strategy: In 2014 the Unit was constrained by a 5-day working week and embryo culture to Day 2 or 3. Additional staffing for 6 day working and additional benchtop and time-lapse incubators enabled extended embryo culture to Day 5 and morphokinetic assessment. The resultant optimised embryo selection lead to greater confidence in SET, as reflected in KPI assessments. A strict mSET policy was introduced in 2017 for all patients with a blastocyst on D5.

Outcomes: The revised strategy resulted in the MPR substantially reducing from 20% to 3.4% without compromising the CPR. Secondary outcomes included an increase in the SET rate (95%) and cumulative pregnancy rate. To reinforce the validity of these outcomes, patient prognosis and laboratory performance was monitored. Patient demographic has remained stable throughout, as illustrated by a multi-factorial patient stratification system (good-prognosis patients: 2014=61%, 2017=63%). Annual cycle numbers and blastocyst formation rate remained constant.

Recommendations: To further optimise the approach, the unit must aim to maintain low MPR but increase CPR. This can be achieved by removing the mSET policy for those patients at low risk of multiple pregnancy.

Conclusion: This synergistic approach has enabled the unit to meet the HFEA MPR target whilst providing the patient with a reliable and safe strategy for achieving a singleton pregnancy.

P170 The significance of blastocoele re-expansion, post warm, on implantation and pregnancy outcomes

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During the process of vitrification and warming, altered osmotic and metabolic conditions could lead to cell damage within the embryo and, depending on the extent of the damage, render the embryo non-viable. Assessing the volume of cell survival within blastocyst stage embryos is subjective but, most commonly, is the only method used by clinics to determine the suitability of an embryo for transfer post warm.

At this clinic, in addition to cell survival, blastocoele re-expansion status is recorded and can be categorised into two groups; re-expansion complete by 2 hours assessment post thaw, and re-expansion incomplete at 2 hours post thaw. The aim of this retrospective study is to determine whether transferring embryos with a re-expanded blastocoele can be correlated with a good prognosis, by comparing implantation, clinical pregnancy and live birth rates between the two groups.

The data set comprises 1797 embryos, thawed and replaced, between January 2016 to October 2017. After comparison, the findings here show that embryos in which blastocoele re-expansion was observed at the 2-hour post-thaw assessment, result in significantly higher rates for implantation, clinical pregnancy and live birth, than those which did not appear to undergo blastocoele re-expansion within 2 hours post warm ($p < 0.01$).

From these data we can conclude that blastocoele activity, post warm, has a significant impact on the outcome of a frozen embryo transfer cycle. One limitation of the study is that the results were not adjusted according to embryo grade at time of transfer. Another is that embryos were not cultured in a time-lapse incubator, which may have provided more accuracy regarding blastocoele re-expansion. The clinical impact of this study is that we may alter the way we counsel patients regarding our expectations of embryos after vitrification and warming.

P171 Time lapse analysis of 1185 human blastocysts resulting in live birth to assess whether gender affects the timing of preimplantation embryo development

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Several non time-lapse studies have reported faster human preimplantation in vitro development of male embryos compared with female, whilst others found no difference. However, studies have been limited by static daily, as opposed to frequent dynamic assessments, possible with time-lapse monitoring. A recently published time-lapse study of 138 blastocysts, found no differences in morphokinetic timings, by gender, whilst another identified two morphokinetic variables differing according to gender^[1,2].

Using EmbryoScope (Vitrolife, Sweden), all embryos were cultured and assessed similarly, using strict and quality assured time-lapse annotation practice and were selected for blastocyst transfer according to standard laboratory protocol. Mean timings for 14 preimplantation morphokinetic variables of 1185 singly transferred blastocysts, from an unselected IVF patient population, resulting in 606 (51%) female and 579 (49%) male babies, were retrospectively compared according to gender reported at birth. T-test was used to assess significance.

Definitions and mean timings $\pm$ sd, in hours post insemination, (female; male) were: Extrusion of second polar body [2.93 $\pm$ 0.93; 2.91 $\pm$ 0.93]; pronuclear appearance [6.79 $\pm$ 1.73; 6.61 $\pm$ 1.72] and fading [22.64 $\pm$ 2.62; 22.71 $\pm$ 2.67]; two to nine cells [t2: 25.40 $\pm$ 3.23; 25.28 $\pm$ 3.05], [t3: 36.07 $\pm$ 3.70; 36.03 $\pm$ 3.55], [t4: 37.15 $\pm$ 3.50; 37.04 $\pm$ 3.70], [t5: 48.32 $\pm$ 5.32; 48.41 $\pm$ 5.09], [t6: 50.21 $\pm$ 4.84; 50.24 $\pm$ 4.70], [t7: 52.23 $\pm$ 5.51; 51.68 $\pm$ 5.34], [t8: 55.18 $\pm$ 6.97; 54.66 $\pm$ 7.17], [t9+66.68 $\pm$ 7.28; 66.17 $\pm$ 6.95]; morula [81.90 $\pm$ 6.68; 81.58 $\pm$ 7.32]; start blastulation [91.35 $\pm$ 5.69; 91.43 $\pm$ 5.86] and full blastocyst [101.25 $\pm$ 5.88; 100.74 $\pm$ 5.98]. 72.2% of female and 69.4% of male babies resulted from ICSI. Mean patient ages delivering either female or male babies were 35.1 $\pm$ 5.24 and 35.45 $\pm$ 5.17 years. No significant differences were found between any of the morphokinetic variables assessed, p values ranged from 0.43 to 0.88. This large retrospective analysis of gender specific human preimplantation development indicates that gender does not impact morphokinetics timings in vitro.

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P172 Lone day 6 (D6) blastocyst vitrification - is it worth it and when?

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Objective: To assess the efficacy of vitrifying lone D6 blastocysts compared to the vitrification of lone day 5 (D5) and all blastocyst vitrification events; regardless of number.

Background: Extended culture can result in very few blastocysts suitable for vitrification by D5/D6. As transfer of fresh embryos on D6, due to delayed blastulation, is associated with lower clinical outcomes^[1] the efficacy of vitrifying lone blastocysts on D6 is questionable.

Study design and results: Retrospective data of clinical pregnancy rate (CPR) of frozen embryo transfer (FET) cycles for patients of all ages, between 01/01/15 and 30/06/18 in one clinic was assessed and analysed statistically using Fisher's Exact test. For lone blastocyst D6 FETs (n=59) compared to single FET D6 irrespective of total number vitrified (n=256) there was a CPR of 22.0% and 30.5% respectively, and no statistically significant difference; p=0.3067. For lone blastocyst D5 FETs (n=101) compared to single FET D5 irrespective of total number vitrified (n=923) there was a CPR of 28.7% and 40.7% respectively, and a statistically significant difference; p=0.0186. Where a single D5 (n=923) or D6 (n=256) blastocyst was replaced in an FET cycle, irrespective of the total number vitrified, the CPR was statistically significantly different (40.7% vs 30.5%); p<0.0001.

Conclusion: D6 single FETs have a similar CPR regardless of number of blastocysts originally vitrified. D5 single FETs however, have reduced CPR when a lone blastocyst was originally vitrified, compared to when multiple blastocysts may have been vitrified. Whilst D5 vitrified blastocysts have a higher CPR generally compared to D6 vitrified blastocysts, the vitrification of D6 blastocysts remains worthwhile regardless of number. However generally D5 vitrification may be preferable, particularly if not vitrified as a lone blastocyst.

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P173 Comparison of genetic analysis between blastocentesis and trophectoderm biopsy: a preliminary study

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Relevance/impact: The practice of pre-implantation genetic testing for aneuploidy (PGT-A) post trophectoderm (TE) biopsy decreases the risk of implantation failure and miscarriages. TE biopsy gives high probability of having informative results when compared to cases in which genetic analysis is performed on either polar body or blastomere biopsy. However, the procedure of biopsy is invasive in nature. A minimal invasive approach being proposed is the analysis of blastocoele fluid known as "blastocentesis", which has demonstrated the presence of genetic material in this fluid^[1,2,3]. This preliminary study compared genomic DNA amplification and chromosomal concordance between blastocoele fluid and trophectoderm cells.

Materials and methods: A total of 21 blastocysts were warmed and cultured for 4-5 hours or until re-expansion. Blastocoele fluid was aspirated from blastocysts as described by Magli et al., (2016). Subsequently, standard TE biopsy was performed on the same blastocyst. Whole genome amplification was performed on the TE biopsy as well as the corresponding blastocoele fluid followed by Next Generation Sequencing (NGS) using MiSeq platform (Illumina, UK).

Outcomes: Twenty-one blastocysts were subjected to blastocoele fluid aspiration. In 11/21 (52.3%) full amplification and 4/21 (19%) sub-optimal amplification was observed. In 6/21 (29%), there was failure of amplification. For the blastocoele fluid with sub-optimal amplification, the low level of DNA was insufficient to proceed with NGS. In the 11 blastocysts with full DNA amplification analysed using NGS, 10 were discordant to the TE biopsy result and the remaining 1 was only concordant for sex chromosomes.

Discussion: In 48% of blastocysts subjected to blastocentesis, informative DNA could not be detected to result in successful amplification. In addition, the amplified DNA from blastocoele fluid was discordant with the chromosomal complement from TE biopsy. Currently, blastocentesis does not seem to be a viable alternative to embryo biopsy for PGT-A.

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P174 Four years of day 4 embryo transfer

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Following risk-benefit analysis, day4 embryo transfer (ET) was introduced into routine clinical practice. The decision to perform ET on day 4 or 5 is made based on clinic logistics and workflow, rather than patient factors. The transfer of one (SET) or two (DET) embryos depends primarily on quality, with SET recommended for high or good quality blastocysts. However, day 4 embryos are less easily graded and are categorised by developmental stage, rather than morphological quality.

Data was analysed from 1353 patients who underwent ET on day 4 (n=209) or 5 (n=1144) using their own eggs, between January 2015 and June 2018. Clinical pregnancy rates (CP/ET) were comparable between day 4 and day 5 ET: Day 4 SET 54.9%; (n=173), day 5 SET 51.6%; (n=803) and day 4 DET 41.7%; (n=36), day 5 DET 39.2% (n=337). Day 4 ET outcome data was analysed according to the developmental stage of the embryos: Blastocyst, Morula and Pre-compaction. On Day 4, 148 patients (70%) had at least 1 blastocyst available and achieved CP/ET of 58.8%, when no blastocyst was available CP/ET was 37.7% (P=0.006) Blastocyst SET or DET on Day4 gave CP/ET of 60.8% (n=125), and 53.3% respectively (n=15) while DET including one blastocyst gave 37.5% (n=8). Multiple pregnancy rate (MPR) was 1.3%, 25% and 33% respectively. Morula SET or DET achieved 43.8% CP/ET (n=32) and 41.9% (n=43) respectively, with one MP. Transfer of Pre-compaction stage gave 33.3% CP/ET (n=18) with 0% MP. These groups were too small to allow statistical analysis.

We conclude that ET on D4 allows a flexible approach to scheduling of ET with clinical results equivalent to D5 ET only when a blastocyst stage embryo is available. However, MP is high and so the transfer of a single embryo should be recommended when the leading embryo has started blastulation.

P175 In a nutshell: Latest updates on gamete and embryo selection from ESHRE 2018

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Sperm, oocyte and embryo selection are essential determinants of the success of an ART programme. With technologies continuously evolving, it is imperative for embryologists to stay up to date with latest developments. The purpose of this abstract is to summarise presentations given at ESHRE 2018.

Information was gathered through the ESHRE abstract book, ESHRE conference app, attendance to lectures, personal communication with authors, and, where lectures clashed and attendance was not possible, through webinar. A total of 300 abstracts were orally presented, out of which 2 (<1%) focused on sperm selection, 3 (1%) on oocyte selection, 19 (6%) on embryo selection (p<0.001), demonstrating a clear bias towards embryo selection research. Sperm selection studies included Artificial Intelligence (AI) as a tool for sperm searching and a redefined MSOME as a predictor of blastocyst formation and

male infertility. Oocyte selection could be improved using follicular size, meiotic spindle morphology and mechanical characteristics.

Embryo selection presentations included genetic (6), metabolic (2), time-lapse (9) and non-time-lapse morphological assessment (2), demonstrating a significant ($p < 0.05$) bias towards time-lapse assessment. Time-Lapse studies focused on quantitative ($n=3$: two studies describing algorithms of prediction of implantation, and one study using algorithms to predict blastulation) and qualitative parameters ($n=6$: three presentations on abnormal cleavages, one on blastomere movement, one on partial compaction, one on blastocyst collapse). AI featured in two of these talks (one in sperm, one in embryo selection). Morphological studies included one study where fast-cleaving embryos had a comparable chance of leading to an euploid embryo than slower cleaving embryos, and one where blastocyst expansion was more efficacious in determining live birth than trophoctoderm and ICM morphology.

In this summary, the scientific strengths and limitations of the findings from these presentations are discussed, within the context of what is already known in peer reviewed publication and clinical applicability.

P176 Case report: Irreversible adherence of a fully hatched blastocyst embryo to the well of an embryoscope slide

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A blastocyst embryo must expand and hatch out from its zona pellucida (ZP) in order to implant into the endometrium. During ART hatched blastocysts must be handled with extra care as fully hatched blastocysts are "sticky" and can adhere to glassware or catheter tubing, which makes them susceptible to damage during handling. This report documents the complete hatching of a blastocyst which adhered to the Embryoscope® slide in which it was cultured.

A 36 year old female and her 40 year old male partner attended the clinic for a cycle of IVF. The female's ovaries were stimulated using a standard stimulation protocol. Oocyte retrieval was performed 36 hours post-hCG, 6 oocytes were retrieved of which 5 normally fertilised. All 5 embryos were cultured in the Embryoscope® using Irvine Scientific's continuous single culture® media.

All 5 developed to the blastocyst stage, 4 of which were suitable for transfer or vitrification. The suitable embryos were vitrified ahead of the transfer and the blastocyst selected for transfer returned to the Embryoslide®. The hatching process allowed the entire blastocyst to free itself from the ZP within a 2 hour period. The hatched blastocyst then adhered to the bottom of the embryoscope slide.

Embryologists were unable to remove the embryo from the slide using stripper tips or glass pipettes. A final attempt was made to detach it using media with double the protein supplement (10% versus 5%), unfortunately the embryo was damaged. The patient's consented to warm one of the vitrified embryos and proceed with an embryo transfer. Following on from this incident lab staff made enquiries with the Embryoscope® slide manufacturers, the culture media providers and the Embryoscope® manufacturers only to find no incidents of its kind had been previously reported. The clinic has now implemented new protocols for the culture/handling of hatching blastocysts.

P177 Retrospective investigation into the effect of ovarian stimulation protocol on embryo quality

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Background: There are 2 primary protocols used for Controlled Ovarian Stimulation (COS) in the UK; GnRH Agonist (long) and GnRH Antagonist. The exact role that COS plays in embryo quality remains unclear. Whilst it is known that agonist cycles yield higher oocyte and embryo numbers, it is not known whether this equates to higher numbers of good quality embryos available for use in treatment.

Aim: The aim of this study was to ascertain whether there was a significant difference in the quality of embryos produced from an agonist protocol compared to antagonist. Since most clinics will cryopreserve only good quality embryos, measurement of quality was indicated by the number of vitrified embryos in a cycle.

Methods: A retrospective analysis of 171 patients (treated between 2014 and 2017) was performed to assess the difference in the number of embryos vitrified between the 2 groups. Only embryos graded 3B/b and above (according to the Gardener and Schoolcraft criteria) were considered suitable for vitrification. Only aetiologies of male factor, tubal factor and endometriosis were included.

Results: There was a significant increase in the number of days of GnRH stimulation and dosage in the agonist group compared to antagonist ($p < 0.0001$). The mean number of; oocytes, MII oocytes, 2PN embryos, cleaved embryos and blastocysts were also significantly higher in the agonist group ($p = < 0.0001$, < 0.0001 , < 0.0001 , < 0.0001 and 0.01 respectively). However, the mean number of vitrified blastocysts between the 2 groups was not statistically significant ($p = 0.396$).

Conclusion: Whilst the agonist protocol was found to achieve higher numbers of both oocytes and embryos compared to the antagonist group, the difference in the number of vitrified blastocysts was not significant. Therefore, it is not beneficial to produce higher numbers of embryos to obtain a better quality yield per cycle.

P178 Freeze all, replace later: A novel strategy to reduce procedural risk and improve clinical outcomes following IVF treatment

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Introduction: The menstrual cycle is affected by exogenous gonadotrophins which are required for superovulation prior to egg collection. A side effect of gonadotrophin use is the risk of ovarian hyperstimulation syndrome (OHSS) which may be life threatening^[1]. Furthermore, published evidence is suggestive that gonadotrophin use may negatively affect uterine receptivity during the stimulated menstrual cycle^[2]. Advances in cryobiology have resulted in blastocyst survival rates in excess of 90% becoming realistically achievable. This has allowed clinicians to recommend freeze all: replace later, as a strategy to minimise the risk of OHSS, without compromising the chances of a positive treatment outcome.

Our laboratory data going back to 2012 indicate a trend towards consistently higher frozen embryo transfer (FET) pregnancy rates from previously vitrified blastocysts when compared to conventional fresh embryo transfer (ET). We believe that by transferring vitrified blastocysts in a natural menstrual cycle the uterus is more receptive to implantation and there is therefore an argument to deploy the freeze all: replace later treatment strategy to a wider group of patients.

Methodology: To test this hypothesis we performed a retrospective data analysis of 173 patients between January 2017 and July 2018 where embryos were cultured using the EmbryoScope timelapse incubator. Clinical pregnancy rates (CPR) were compared between the two groups for three age ranges; <35, 35-39 and >40.

Results: A trend in favour of CPR following FET was observed in each age group, (<35: 73.3% vs 45.9%, 35-39: 57.9% vs 41.3%, >40: 40.0% vs 32.4%, overall: 61.5% vs 40.3%). Statistical significance was achieved in the overall dataset (p=0.018).

Conclusions: We believe that there is clinical benefit in offering the freeze all: replace later strategy to a wider group of patients. It is important that patients are thoroughly counselled regarding the implications of this strategy and the fact that it may not be appropriate to everyone prior to treatment.

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P179 Does EmbryoGlue® improve outcomes for fresh and frozen embryo transfers?

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The objective of this study was to determine whether using EmbryoGlue® results in an improvement in IR and CPR in fresh ETs and Frozen Embryo Transfers (FETs) in patients with at least one previous ET. This retrospective data analysis included 120 patients that underwent 134 fresh ETs and 542 patients that underwent 616 FETs between June 2016-2018.

Data was divided into fresh and frozen transfers using EmbryoGlue® or Sage 1-Step™ culture media. IR and CPR were compared. To calculate statistical significance, Fisher's Chi-square exact test and t-test were used and P<0.05 was considered to be statistically significant. EmbryoGlue® is thought to improve implantation of embryos. Determining its effectiveness will provide the evidence on whether it should be routinely used to improve outcomes or whether it is an add-on that should be re-considered before being offered to patients.

IRs and CPRs were comparable across all groups for both fresh and frozen cycles. The CPR for fresh D5 ETs using EmbryoGlue® was slightly higher compared to the no-EmbryoGlue® group (45.7% vs. 44.1%, respectively), though not statistically significant. The average ages at the time of egg collection for both groups were comparable. In the frozen data, the average age at the time of freeze was significantly higher in the EmbryoGlue® group compared to no-EmbryoGlue® group (33.9 vs. 33.1, respectively, P<0.01), which might be due to different group sizes.

This study demonstrates that the use of EmbryoGlue® does not have an impact on cycle outcomes. The slightly higher CPR in the fresh D5 EmbryoGlue® group needs to be confirmed with further data. The significantly higher average age in the frozen EmbryoGlue® group would suggest that EmbryoGlue® has been recommended for advanced age. Due to the uneven group sizes, further data should be collected to determine the impact on IR and CPR.

P180 The use of Primo Vision time-lapse system to assess early embryo morphokinetic parameters and their association with embryo implantation potential

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Background: There is contradicting evidence on whether early embryo cleavage timings can help select the embryo with the greatest implantation potential^[1]. This study uses the Primo Vision™ time-lapse system (TLS) (Vitrolife, Sweden) to evaluate whether these events are predictive of implantation success. These results could help increase success rates and reduce multiple births.

Methods: This study included 133 embryos with known implantation data (KID). Cycles were included if the female patient was <37 years old and had a single embryo transfer. The outcome was either successful implantation (KID+ve), detected by the presence of a fetal heart beat at the 7 week scan, or failed implantation (KID-ve), indicated by a negative hCG test. Using the Primo Vision™ TLS, the time from insemination to the 2-cell stage (t2), 3-cell stage (t3) and 4-cell stage (t4) as well as the time intervals between the first and second division (cc2) and the second and third division (s2), were recorded (in hours) for each embryo transferred. The embryo cleavage timings within the KID+ve and KID-ve groups were statistically analysed to identify any association with implantation success.

Results: The KID+ve embryos had significantly shorter s2 times (p=0.0091). Logistic regression identified that neither age, OR=1.00 (95% CI 0.89-1.12, p=0.969) nor treatment type, OR=0.99 (95% CI 0.47-2.06, p=0.968) were associated with implantation potential, however the cleavage timings t4, OR=0.44 (95% CI 0.24-0.84, p=0.013) and s2, OR=0.44 (95% CI 0.23-0.84, p=0.013) were predictors of implantation. ROC curve analysis for s2 gave an area under the curve value of 0.635 (95% CI 0.547-0.717). **Conclusions:** The cleavage timings, s2 and t4, were associated with implantation potential. However as these timings are poor predictors, they should only be considered to help de-select embryos, with conventional embryo grading used for the final clinical decision.

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P181 Time-lapse parameters as indicators of cryopreservation potential during human preimplantation embryo development

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Background: Given the growing trend towards single embryo transfer, it is of high clinical importance to be able to select spare embryos with the best developmental potential for cryopreservation.

Aim: To investigate whether time-lapse (TL) data can improve selection criteria for cryopreservation.

Methods: This study looked at 160 patients who underwent fresh autologous treatment at St Mary's Department of Reproductive Medicine and whose embryos were cultured in the EmbryoScope® system. Retrospective TL data was gathered for embryos cryopreserved on day 5 (D5vit; n=210), day 6 (D6vit; n=195) and discarded poor-quality blastocysts (Disc; n=210). The capacity of TL parameters to predict allocation of embryos into one of these three groups was assessed with the use of receiver operating characteristic (ROC) analyses.

Results: ROC analysis of TL parameters showed time to start of compaction (tSC), start of blastulation (tSB) and blastocyst formation (tB) to have the highest capacity to discriminate between D5vit and D6vit embryos. None of the parameters could distinguish a D6vit from a Disc embryo. Based on these findings we developed a hierarchical classification model incorporating threshold values for tSC, tSB and tB, to categorise embryos into eight groups with decreasing cryopreservation potential on D5 of embryo culture.

Conclusion: Our findings propose specific TL parameter threshold values for the identification of blastocysts suitable for cryopreservation on D5. The predictive capacity of these TL parameters is likely to be limited and future work will need to identify D6vit and/or Disc embryos on D5 and to clinically validate the hierarchical classification model.

P182 Embryo quality of each embryo transferred as a measure for optimum outcome in a double embryo transfer: A cohort study for optimal success whilst limiting the multiple pregnancy rate

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Background: The HFEA single embryo transfer (SET) initiative, aims to reduce the multiple pregnancy rate (MPR). This study aims to determine which embryo quality combinations improve outcome whilst limiting the MPR in age-specific women.

Methods: Retrospective cohort study of all embryo transfers (ETs), from 2010-2018, stratified by number of ET (single/double, [SET/DET]), stage (cleavage/blastocysts), embryo quality (two good/one good+borderline/two borderline) and female age (<37/37-39/40-42/>42 years), analysed for singleton live birth rate (SLBR) and MPR.

Statistical significance: p<0.05 (Chi-square).

Results: 16440 ETs analysed: 7885 cleavage stage and 2353 blastocysts; 2474 excluded (incomplete data). Across all ages, a blastocyst DET of: two good embryos resulted in a 29% SLBR and 22% MPR; a good+borderline embryo resulted in a 29% SLBR and 16% MPR; and, two borderline embryos resulted in a 22% SLBR and 3% MPR; $p=0.00952$. During the same time period a SET of a good blastocyst resulted in a 38% SLBR and 1% MPR, whilst borderline embryos had a 20% SLBR; $p=0.00268$. Whilst, a cleavage stage DET of: two good embryos resulted in a 25% SLBR and 8% MPR; a good+borderline embryo resulted in a 20% SLBR and 5% MPR; and, two borderline embryos resulted in a 22% SLBR and 3% MPR; $p=0.2999$. Women <37 , demonstrated no difference between the SLBR after the transfer of two good or two borderline blastocysts (29% vs 28%), but a significant difference between the MPR (25% vs 2%, respectively; $p=0.004133$). Women between 37-39 years had similar trends with two good embryos (29% SLBR and 23% MPR), whilst a good+borderline had a 34% SLBR and 11% MPR.

Conclusions: A DET should only be considered with two borderline blastocysts and/or in women >37 years, optimising the SLBR without increasing the MPR. A DET of two borderline blastocysts or two good quality cleavage embryos can achieve an SLBR of $>25\%$ with $<10\%$ MPR.

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P183 Controlled follicle flushing significantly improves pregnancy rates in IVF/ICSI cycles

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Background: Controversy regarding the role of follicle flushing on pregnancy outcomes is ongoing. The current literature does not distinguish between manual and mechanical flushing and this may be the largest contributor to reported outcomes. We have assessed the result of conventional manual follicle flushing versus flushing using a temperature and flow rate controlled system.

Methods: Retrospective analysis of IVF/ICSI cycles in a large teaching hospital between January 2016 and December 2017 where follicle flushing was used at egg collection.

Results: A total of 838 IVF/ICSI cycles were analysed: 452 cycles incorporated manual flushing (Group 1) and 386 underwent controlled flushing (Group 2). No significant difference was demonstrated in the demographic data between the two groups (female age [36 (IQR 32-38) vs 36 (IQR 33-39), $p=0.657$], duration of stimulation [12 (IQR 11-14) days vs 12 (IQR 11-14) days, $p=0.625$] and total dose of FSH used [3300 (IQR 2100-4200)iu vs 2925 (IQR 1950-3900)iu, $p=0.230$]). Group 2 demonstrated a significantly higher oocyte yield (number of oocytes retrieved per number of follicles present at the last stimulation scan) (88% [4478/5070] vs 84% [3375/3993], $p<0.05$). However, a lower oocyte maturity rate was demonstrated with Group 2 (72% vs 85%, $p<0.05$). No difference was seen in the fertilisation rate (57% vs 63%, $p=0.169$), but Group 2 did demonstrate a significantly higher implantation rate (52% vs 41%, $p<0.05$) and clinical pregnancy rate (42% vs 32%, $p<0.05$). To further assess the impact of oocyte maturity, we analysed ICSI-only cycles. In this subgroup, the higher implantation (54% vs 43%, $p<0.05$) and clinical pregnancy (47% vs 34%, $p<0.05$) rate were maintained in Group 2.

Conclusions: This study demonstrates that strict control of follicle flushing does have a significant impact on clinical pregnancy rates in comparable patients. Controlled flushing is better able to sustain oocyte competence, resulting in significant improvements in pregnancy rates.

P184 Second polar body morphokinetics are not associated with embryo quality or miscarriage

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Background: Embryo morphokinetics provide a large amount of data of early embryonic development. Timing of extrusion of the second polar body has been shown to be highly predictive of oocyte fertilisation with conflicting data on embryo quality. We analysed embryonic morphokinetics for ICSI-only cycles to assess whether timing of second polar body extrusion is associated with embryo quality or miscarriage.

Methods: This was a retrospective analysis of ICSI cycles using time-lapse in a large teaching hospital between January 2012 and December 2016. All included patients had a single fresh day 5 embryo transfer that resulted in a positive pregnancy test.

Results: We analysed 206 cycles. There was a significant correlation between age and embryo quality ($p<0.05$) but no correlation between timing of extrusion of polar body and embryo quality ($p>0.05$) even when controlling for age ($p=0.641$).

There was a correlation between age and miscarriage ($p < 0.05$) but no correlation between miscarriage and timing of second polar body extrusion ($p = 0.369$) or embryo quality ($p = 0.074$) after controlling for other variables.

Additionally, we looked at cycles where patients had a blastocyst transfer ($n = 168$) only. There was no correlation between embryo quality and age ($p = 0.087$) or timing of extrusion of the second polar body ($p = 0.654$). There was a significant association between age and miscarriage ($p < 0.05$) but no significant correlation between miscarriage and timing of second polar body extrusion ($p = 0.703$) or embryo quality ($p = 0.965$) after controlling for other variables.

Conclusion: Our data shows that timing of second polar body extrusion is not predictive of embryo quality or of miscarriage.

P185 The effect of oxygen tension on blastocyst culture in an oocyte donation programme: A randomised controlled equivalence trial

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Background/objectives: The oocyte donation model represents an accurate method to assess blastocyst culture and clinical outcome under different oxygen tensions, e.g. atmospheric-O₂ (20%) versus low-O₂ (5%). Animal studies have demonstrated the beneficial effects of culturing embryos under low O₂. Nevertheless, many prospective randomised human trials have shown controversial results^[2,3]. While some studies have shown comparable results between both O₂-groups, others have shown detrimental effects related to the adverse influence of reactive oxygen species (ROS)^[4,5]. The objective of this study was to compare embryonic development parameters along with clinical endpoints between two oxygen tensions in oocyte donation cycles.

Materials and methods: In this prospective, randomised, equivalence trial, embryo quality (blastocyst stage transfers on day 5/6) and clinical outcomes between two oxygen tensions were compared. Primary outcome was clinical pregnancy rate (CPR). Study was carried out at 3394 meters above sea level equating to an O₂ tension of 17.7%.

Results: Preliminary analysis of this pilot study included 132 patients allocated to both O₂ groups. Sample size was estimated based on an expected 15% CPR increase with power of 80% and α -error of 0.05. The CPR and implantation rates were similar with atmospheric-O₂ and low-O₂ (44% vs 42% $P = 0.78$ and 44% vs 39% $P = 0.47$ respectively). Higher proportion of top-quality blastocysts was observed in low O₂ group (69% vs 73% $P = 0.49$), consequently, higher cryopreservation rate was achieved (7.9% vs 14.5% $P = 0.24$).

Discussion/conclusions: Embryo culture under low O₂ is justifiable and mitigates the detrimental effects caused by ROS. Despite similar pregnancy rates, improved blastocyst quality and higher cryopreservation rates were achieved with low-5% O₂, although this was not statistically significant. At high altitudes, reduced barometric pressure may have a beneficial role. Calculation of cumulative pregnancy rates and live birth rates will provide better understanding about the effect of reduced O₂.

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P186 Does trophectoderm biopsy of fully hatched blastocysts affect implantation potential?

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Objective: To examine the outcome (survival, implantation and clinical pregnancy rates; SR, IR, CPR) of vitrified fully hatched blastocysts with or without trophectoderm biopsy immediately prior to vitrification. A retrospective analysis was performed of PGD and non-PGD cycles in which single blastocysts were warmed and transferred between June 2014 and January 2018.

Methods: Following IVF/ICSI, embryos were cultured in single-step SAGE media (Origio, Denmark) in an atmosphere of 5%O₂ and 6%CO₂. For PGD, laser-assisted zona ablation was performed on Day3 post ICSI; fully hatched blastocysts were biopsied on Day5/ Day6 and vitrified immediately. Vitrification of PGD and non-PGD hatched blastocysts was carried out using Cryotop[®] and Kitazato reagents (Dibimed, Japan). Blastocysts were warmed and transferred in frozen embryo transfer (FET) cycles.

Results: A total of 61 FET cycles for 59 patients were included in the analysis, 43 for PGD and 18 for routine IVF/ICSI treatment. Although female age was significantly higher in the non-PGD group compared to the PGD group (37.1 vs 33.3; $p=0.0002$), there were no significant differences between the two groups in mean anti-Mullerian hormone (AMH; 30.12 vs 18.51; $p=0.0724$), or in mean number of oocytes collected (15.9 vs 13.0; $p=0.2013$), mature oocytes (13.1 vs 12; $p=0.5927$), fertilised oocytes (9.9 vs 9.0; $p=0.5644$) and the blastocyst quality (chi-squared=5.8179; $p=0.054$). While the survival rate (SR) of biopsied hatched blastocysts was significantly higher than that of non-biopsied hatched blastocysts (95.3% [41/43] vs 50.0% [9/18]; $p=0.0001$), there were no significant differences in implantation rate (IR) (30.2% [13/43], 22.2% [4/18]; $p=0.7552$) and clinical pregnancy rate (CPR) per embryo warmed (30.2% [13/43] vs 22.2% [4/18]; $p=0.7552$).

Conclusion: Although the numbers are small, this is the first report comparing outcomes of FET cycles using warmed/vitrified biopsied and non-biopsied fully hatched blastocysts, and suggests no adverse effect of trophectoderm biopsy on fully hatched blastocysts.

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P187 Inverse ratios of T lymphocyte subpopulations in peripheral blood and the endometrium of women with recurrent pregnancy loss and implantation failure

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Introduction: A significant challenge faced in reproductive medicine is recurrent pregnancy loss (RPL) and implantation failure (IF). Difficulties achieving pregnancy places significant psychosocial, emotional and economical strain on a couple^[1]. There are many causes of miscarriage, but in over 50% of cases of pregnancy loss the underlying aetiology is not clear^[2]. This study examines T lymphocyte populations in the blood and endometrium for patterns that may play a role RPL and IF.

Methods: Endometrial biopsies and peripheral blood samples of women with RPL and IF were collected. T lymphocyte subpopulations were analysed in 612 endometrial samples and 332 blood samples.

Results: In peripheral blood, CD4+ T cells are dominant, with a CD4:CD8 ratio of 2.2. This is reversed in the endometrium, where CD8+ cells dominate, with a mean CD4:CD8 ratio of 0.81. Interestingly, when CD4+ T lymphocytes are divided into subsets, marked differences are noted between the two environments. Blood CD4+ cells are primarily of the T helper (Th)-2 subtype, comprising almost half of this group (46.7%), but Th1 cells still constitute a sizable segment of this population (25.1%), giving a relatively balanced Th1:Th2 ratio of 0.66. The local environment in the uterine cavity is entirely different. Here, the Th1 population is almost seven times higher than that of Th2 cells (66.9% vs 9.7%) giving a very skewed Th1:Th2 ratio of 17.3, a major change from that found in the peripheral circulation.

Conclusion: RPL and IF exhibit inverse CD4:CD8 ratios between the blood and endometrium. Th1 levels are significantly raised in the endometrium of women with RPL and IF. Comprehensive understanding of these patterns could contribute to higher success rates in ART, or development of novel treatments for recurrent miscarriage, by the development of appropriate and personalised immunotherapy, guided by detailed and validated testing.

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P188 Post-thaw embryo intactness does not impact on obstetric outcomes in frozen embryo transfer cycles

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Purpose: With increasing numbers of frozen embryo transfer (FET) cycles and advances in embryo cryopreservation, embryo intactness has been explored as a potential predictor of treatment success. The impact of post-thaw intactness on pregnancy birth rates is unclear with even less known about its effect on obstetric outcomes. We aimed to ascertain whether post-thaw percentage embryo survival influences obstetric and perinatal outcomes.

Methods: This was a retrospective analysis of consecutive FET cycles between January 2013 and January 2016. Post-thaw percentage survival of day 5 and 6 embryos was recorded along with obstetric outcomes. We included single embryo transfer (SET) and double embryo transfers (DET). Obstetric outcomes were analysed according to percentage of post-thaw embryo survival. This was categorised into group A (90-100% survival), B (80-90% survival) and C (<80% survival).

Results: We examined 249 FET pregnancies that resulted in pregnancy. Obstetric outcomes were analysed in accordance to the number of embryos transferred, with singleton and twin pregnancies analysed separately to avoid bias. In SET (n=95) singleton pregnancies, there was no significant association between intactness and gestational age ($p=0.970$), birth weight ($p>0.05$), mode of delivery ($p=0.422$) or low birth weight ($p=0.448$). In DET singleton pregnancies (n=106) there was no correlation between intactness and gestational age ($p=0.430$), birth weight ($p=0.504$), pre-term birth ($p=0.133$) or mode of delivery (0.306). In DET twin pregnancies (n=48), there was no correlation between intactness and gestational age ($p=0.428$), birth weight ($p=0.618$), pre-term birth ($p=0.577$) or mode of delivery ($p=0.306$).

Discussion: Whilst blastocyst survival plays an integral role in implantation and continued pregnancy, our study demonstrates that once pregnancy is established, post-thaw embryo survival does not negatively impact on obstetric outcomes.

P189 Investigating the safety of laser-assisted hatching procedures on the mouse embryo

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Background: A mammalian embryo must implant into the endometrium to generate a successful pregnancy^[1]. While embryo implantation failure is the rate-limiting step in the attainment of pregnancy, little is known of the etiology of this condition^[2,3]. Laser-assisted embryo hatching (LAH) has been suggested as a procedure to facilitate embryo implantation^[4]. However, current literature relating to the efficiency of LAH is highly inconsistent^[5-7] and there is a significant paucity of research relating to potential effects at the molecular level. The aim of this study was to assess the safety of these LAH methods to uncover detrimental or beneficial effects of laser treatment on the embryo.

Methods: Experiments involve three LAH treatments (A: complete LAH (the ZP was completely breached); B: partial LAH (the ZP was partially thinned with intact inner membrane); C: zona thinning (extensive ZP was thinned)) and one control group (D). Following treatment on the day of collection or thawing (8-cell embryo stage), embryos were cultured for 2 days and then assessed from four different aspects, including embryo developmental assessment, cell apoptosis (DNA fragmentation and Caspase activity), transcript level of key genes, and stress response (HSP70).

Results: Data showed that the hatching/hatched rate was significantly higher in the complete LAH group (95.8%, 66.7%, 56.4% and 61.8% for groups A-D; $P<0.001$). As for cell apoptosis, there were no significant differences in terms of apoptosis rate and caspase activity. Additionally, HSP70 staining and the expression of Hspa1a showed that no stress response was induced by LAH treatment. However, RT-PCR results showed that Bax transcript level decreased in the complete LAH treatment group, while other genes showed no differences.

This work provides preliminary evidence for the safety of LAH application. However, future work will be conducted using microarrays and 3D modelling implantation model to explore how LAH might influence embryos' gene expression and implantation.

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P190 The effect of prokineticin 1 on the porcine conceptus during implantation process

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Prokineticin 1 (PROK1), also called as endocrine gland-derived vascular endothelial growth factor (EG-VEGF) acts via two G-protein coupled receptors: PROKR1 and PROKR2. Among other factors PROK1 controls angiogenesis and cell proliferation. Important role for PROK1 has been implicated in processes related with establishment of pregnancy in human. The aim of the present study was to determine *PROK1* and *PROKR1* genes expression in the porcine conceptus and to evaluate the effect of PROK1 on the conceptus cells proliferation and adhesion.

RNA was isolated from porcine conceptuses recovered from uteri of gilts on days 10-12 (n=4), 14-16 (n=7), 17-19 (n=4) and 20-25 (n=4) of pregnancy and reverse-transcribed to cDNA. Gene expression was determined by real-time PCR. Conceptuses

collected on day 15 of pregnancy (n=10) were digested with trypsin for 30 min at 37 °C. Released conceptus cells were then cultured until 60-70% confluency. Confluent cells were treated with vehicle or 40 nM PROK1, with/without 1 µM of PROKR1 antagonist (PC7) (proliferation and adhesion assays) and 25 µM PD098059, 25 µM LY294002, 2.5 µM Akt1/2 kinase inhibitor, 25 µM SQ22536, 1 µM Bay 11-7082 (only in proliferation assay) for 24 h. Statistical analyses were performed using one-way ANOVA, followed by Tukey (gene expression and cell adhesion) and Dunnett's Tests (cell proliferation).

Expression of *PROK1* was significantly greater in the conceptuses during implantation period (days 17-19) and placenta development (days 20-25) than in pre-implantation period (days 10-12) ($p<0.05$). No differences in mRNA content of *PROKR1* in the investigated days were found. PROK1 significantly elevated conceptus cell proliferation ($p<0.05$) and adhesion ($p<0.05$). Co-treatment with PC7 and inhibitors diminished stimulating effect of PROK1.

Results of the present study indicate that PROK1 and PROKR1 are important factors involved in the porcine conceptus implantation and placenta development processes.

P192 Does intracytoplasmic sperm injection improve fertilisation rate and pregnancy outcome, compared with IVF, in patients with non-male factor infertility when 3 or fewer oocytes are retrieved?

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The literature is not in agreement as to whether insemination method should be altered, from IVF, in cases of low oocyte yield in order to increase the fertilisation rate and pregnancy outcome. A randomised controlled trial^[1] in couples with non-male infertility, demonstrated that ICSI does not yield better reproductive outcome but in other publications^[2,3] a higher fertilisation rate was found with ICSI in cycles of low oocyte yield.

In the present study, data was retrospectively analysed from 980 cycles (November 2012-October 2017) with ≤ 3 oocytes retrieved in non-male factor infertility cases. Literature search was performed in PubMed, using search criteria "IVF, ICSI, poor responder, oocyte number". Cycles were classified into two groups by different insemination technique: IVF group (n=926), and ICSI group (n=54). Fertilisation rate per mature oocytes (MII oocytes), positive human chorionic gonadotropin (hCG), clinical pregnancy (CP), implantation rate (IR) and live birth (LB) per embryo transfer rate were recorded and statistically analysed with chi-squared test. A p value of <0.05 was considered statistically significant.

The IVF group presented a significantly higher normal fertilisation rate per mature oocytes compared with the ICSI group (74.71% vs 64.81%, respectively, $p=0.02$). The CP (22.05% versus 18.92%), IR (27.53% versus 20.51%), and LB rate per transfer (18.38% versus 16.22%) had a favourable trend towards IVF, but no statistically significant difference was observed, compared with the ICSI group. Previous cycles, female indication, patient age, embryo quality, previous pregnancies and indication for fertilisation method have not been controlled.

This study is in agreement with the majority of the published studies demonstrating that ICSI does not improve outcome for patients when ≤ 3 oocytes are retrieved. Although randomized trials are needed to confirm this study's findings, we propose that the choice of fertilisation technique should not be based on oocyte number.

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P193 Double the chances of conception in first cycle of use with new connected ovulation test

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Introduction: Natural conception requires intercourse during the fertile period of the cycle and home ovulation tests are a convenient method for identifying the fertile period. Most tests only detect luteinising hormone to predict impending ovulation. Clearblue Connected Ovulation Test System (COTS) also detects estrone-3-glucuronide to identify the wider fertile phase. The ovulation test syncs via Bluetooth® to an app on the user's phone, which also provides guidance on testing. Accurate timing of intercourse should maximise chances of pregnancy, but there have previously been no randomized, controlled studies examining efficacy of home ovulation tests. The aim of this study was to demonstrate that using the Connected Ovulation Test System increases chances of pregnancy.

Methods: UK Women seeking to conceive, aged 18-40, were randomized into 2 groups; the test group used COTS, whereas the control group were asked not to use ovulation tests. The study lasted for up to 2 cycles. Volunteers were required to conduct digital home pregnancy tests and collect urine samples at the end of each cycle, and these were returned to the

study site. Urinary hCG was measured in the urine samples by AutoDELFIA. This information combined with diary recording of menses was used to determine pregnancy status at the end of each cycle.

Results: The one-cycle pregnancy rate was 14.7% for the control group (n=402) and 25.4% (n=378) for the test group; Odds Ratio 2.0 (95% CI 1.38-2.84). The two-cycle pregnancy rate was 28.6% for the control group and 36.2% for the test group; Odds Ratio 1.4 (95% CI 1.04-1.91).

Conclusions: Women using COTS had twice the odds of becoming pregnant in first cycle, compared to women not using ovulation tests. This is the first randomised, controlled study to demonstrate the efficacy of a home ovulation test. This means that providing accurate information on their wider fertile window, with connectivity to their phone, can provide real benefit to women seeking to become pregnant.

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