

ORAL PRESENTATIONS

SRF POST DOCTORAL PRIZE SESSION

SRF.1 Tgfb β /smad signaling regulates granulosa cell proliferation and tzp generation during early follicular development

Granados Aparici Sofia; Yang Qin; Clarke Hugh J.

Research Institute - McGill University Health Centre; McGill University; Montreal, Quebec, Canada

During initiation of follicular development, the somatic cells surrounding the oocyte, termed granulosa cells (GCs), start to proliferate and cuboidalise. Shortly after, an extracellular layer called the zona pellucida starts to form and physically separates the oocyte from the GCs. Since GC-oocyte contact-dependent communication is essential for oocyte development, GCs generate transzonal projections (TZPs), filopodia-like structures that penetrate the zona pellucida and establish contact with the oocyte plasma membrane. Since the TGF β superfamily of ligands have a well-known role in granulosa function in growing follicles, we sought to investigate whether the canonical mediators, the SMAD3/4, regulate the rate of initial GC proliferation as well as the generation of TZPs in preantral follicles. Quantitative RT-PCR and immunofluorescent histological sections of mouse ovaries showed expression and localisation of Smad3/4 in GCs of preantral follicles from non-growing stage onwards. The tamoxifen-inducible Cre-ER/floxed Smad4^{+/+} knockout (KO) system was used in neonatal ovaries and granulosa cell-oocyte complexes (GOCs) in culture to delete the Smad4 gene in non-growing and growing follicles. Whole ovaries and GOCs were exposed to tamoxifen (1 μ g/ml) for 1 and 2 days, respectively, followed by additional days in tamoxifen-free conditions. Wholmount staining of isolated follicles and GOCs was performed and confocal images were taken to quantitatively analyse the GC number, oocyte diameter and TZP number using Image J software. Single-layered follicles isolated from Cre⁺ ovaries after culture showed a significant decrease in GC number per oocyte diameter when compared to Cre⁻ controls. Cre⁺ GOCs showed a significant decrease in the number of TZPs whereas oocyte diameter remained unchanged. Our results suggest a new role for the canonical SMAD signalling on the molecular mechanisms regulating early GC proliferation and germ line-somatic communication in preantral follicles which may help identify novel markers of follicle/oocyte quality and provide insight into the causes of infertility.

SRF.2 Differential follicle stimulating hormone glycosylation modulates follicle growth and survival rates

Johnson Gillian P¹; Bousfield George R²; Hardy Kate³; Jonas Kim C¹

¹King's College London; ²Wichita State University, Kansas; ³Imperial College London

Ovarian ageing is a naturally occurring physiological process, marked by dynamic changes in ovarian function and hormone secretion. Several pathologies are associated with ovarian senescence, including; osteoporosis, diabetes, cardiovascular disease and impaired cognitive function, therefore, understanding the physiological processes regulating this is imperative for identifying novel treatments. A key endocrine regulator of ovarian function is the heterodimer glycoprotein hormone, follicle stimulating hormone (FSH). FSH is secreted as two glycosylation variants; partially glycosylated FSH (FSH21) and fully glycosylated FSH (FSH24). These variants have different bioactivities, with FSH21 more biologically active than FSH24. Interestingly, the ratio of FSH21:FSH24 changes with age, with FSH21 predominant in women of reproductive prime, and FSH24 predominant in menopausal women. Yet, if these FSH glycosylation variants differentially modulate follicle growth and survival remains unknown. This study therefore aimed to determine the effects of FSH21 and FSH24 on follicle growth and survival. To do this, mouse ovarian follicles were isolated from 3-4wk-old-C57/BL6 mice and treated +/- 10ng/ml, FSH21(n=74), FSH24(n=66), a ratio of FSH21:FSH24 at 80:20 (to mimic reproductive prime; n=68) or FSH21:FSH24 at 20:80 (to mimic late peri-menopause; n=66). Follicles were cultured for up to 96hrs and imaged daily to evaluate follicle morphology. Follicle growth was markedly increased at all time points, when cultured in the presence of FSH21 or 80:20 FSH21:FSH24, in comparison to control, FSH24 alone and 20:80 FSH21:FSH24 conditions. Follicles treated with FSH24 or FSH21:FSH24 at 20:80 tended to undergo basement membrane rupture and oocyte extrusion. Moreover, survival rates were significantly decreased in follicles treated with FSH24 or FSH21:FSH24 at 20:80. These data suggest that the nature of FSH glycosylation modulates the follicular cellular environment to regulate follicle growth and survival. These findings have important implications for IVF ovarian hyperstimulation treatment regimens. Moreover, the ratio of FSH21:FSH24 may be an important novel biomarker of ovarian ageing.

SRF.3 Exploration of chemerin system in human granulosa cells: involvement in polycystic ovarian syndrome

Estienne Anthony; Mellouk Namya; Bongrani Alice; Ramé Christelle; Guérif Fabrice; Froment Pascal; Dupont Joelle

INRA

Chemerin is an adipokine involved in several metabolic processes. It binds three G protein-coupled receptors: CMKLR1, GPR1, and CCRL2. Recently, a role for the chemerin system in ovarian follicle function and steroidogenesis has been reported in mammals. In both human primary granulosa (hCGs) and human ovarian granulosa-like tumour cell line (KGN), chemerin inhibited IGF-1-induced steroids production. Thus, we investigated chemerin system in hCGs from PCOS patients and KGN and modulation of its signaling activities using a CMKLR1 nanobody (C4910). Our study showed that chemerin mRNA was highly expressed in hCGs of obese, PCOS-O and ECHO-O patients as compared to control, PCOS and ECHO patients and we observed opposite profile for CMKLR1. The mRNA expression of CCRL2 was lower in PCOS, obese and ECHO-O patients as compared to control, ECHO and PCOS-O. In parallel, cultured KGN cells and hCGs were stimulated or not with human recombinant chemerin and/or with C4910. We found that in KGN cells CMKLR1 and CCRL2 receptors formed a heterodimer after 5 minutes of stimulation with chemerin and gradually increased until 60 minutes. We also showed that chemerin rapidly activates MAPK ERK 1/2, P38 and Akt phosphorylation and more slowly AMPK and β -arrestin phosphorylation in KGN cells. Experiments on KGN cell co-incubated with chemerin and the C4910 showed a lower phosphorylation of P38 after 5 minutes than in cells only incubated with chemerin. Experiments on KGN and hCGs co-incubated with chemerin and C4910 showed a reduced progesterone production with a reversal effect of co-incubation with C4910. Our results indicated that chemerin system is expressed in granulosa cells and a deficit of CMKLR1 seems to protect against follicular development disruptions in PCOS patients. We also described the chemerin signaling involved in inflammation and steroidogenesis that was partially blocked by a potential therapeutic nanobody targeting CMKLR1.

SRF.4 Aberrant Igf2-H19 expression in the placental endocrine zone increases the susceptibility of the mother to poor metabolic health

Lopez-Tello Jorge; Yong Hannah EJ; Christoforou Efthimia R; Napso Tina; Sandovici Ionel; Constancia Miguel; Sferruzzi-Perri Amanda N

Centre for Trophoblast Research

During pregnancy, the mother must adapt metabolically to support offspring growth. Failures in maternal metabolic adaptation can result in gestational diabetes, which is a risk factor for type-2 diabetes in later life. The placenta secretes hormones with metabolic effects, although the precise role of its endocrine function in determining maternal health is largely unknown. Previous work has shown that the imprinted Igf2-H19 locus is involved in controlling placental endocrine function and conceptus development in mice. This study used conditional mis-expression of the Igf2-H19 locus, through deletion of the imprinting control region ICR1, to induce placental endocrine malfunction and study its consequences for maternal metabolism during and after pregnancy.

Mice were crossed to produce entire litters with reduced levels of the H19 gene and activation of the normally silent maternal Igf2 gene in the placental endocrine zone (H19DMRflox/TpbpaCre; Jz-ICR1 Δ). On day 16 of pregnancy, dams were terminally anaesthetised for blood collection and tissues collected for molecular analysis. Other dams delivered and, at eight weeks post-partum, they underwent a glucose tolerance test followed by tissue collection one week later. Data were compared to dams with unaltered placental Igf2-H19 locus expression.

Jz-ICR1 Δ dams had heavier kidney, heart and liver weights compared to pregnant controls. They also displayed higher circulating levels of glucose, insulin, leptin, progesterone, estradiol and corticosterone. Proteins involved in glucose handling (e.g. PI3K-p110 α , total AKT and p70S6K) were downregulated in the liver of Jz-ICR1 Δ dams. The placental endocrine zone was increased by Jz-ICR1 Δ , but maternal plasma IGF2 and fetal growth were unchanged. Dams that carried Jz-ICR1 Δ placentas had lower levels of adiposity and were glucose intolerant at 8-9 weeks after delivery.

In conclusion, genetically-induced expansion of the placental endocrine zone alters maternal body composition and metabolic and endocrine state during pregnancy with consequences for the postpartum health of the mother.

SRF.5 The impact of paternal diet on male reproductive physiology and fetal development in mice

Morgan Hannah L¹; O'Neil Donna²; Dunn Warwick²; Watkins Adam J¹

¹University of Nottingham; ²University of Birmingham

Background: There is evidence that poor paternal diet increases the likelihood of offspring developing cardio-metabolic disorders as adults. This study investigated how sub-optimal paternal diet; with/without supplementation of essential vitamins and minerals; impacts male reproductive physiology and fetal development.

Methods: Male C57BL/6 mice were fed one of five diets from 7 weeks old for at least 8 weeks; normal protein (NPD (18% casein)), low protein (LPD (9% casein) or western diet (WD (20% fat, 0.15% cholesterol)) or diets supplemented with methyl-donors (MD-LPD and MD-WD). Males were mated with 8-12 week-old C57BL/6 females maintained on standard chow prior to, and during, pregnancy. On embryonic day 17.5 fetal and placental weights were recorded. One-carbon metabolite levels in stud male liver and testes were determined by targeted metabolomic analysis using gas chromatography-mass spectrometry.

Results: Paternal diet had no impact on fertilisation rate or litter size. Fetal weight correlated negatively with litter size in all diet groups except WD and MD-WD. Furthermore, paternal WD and LPD impacted the distributions of fetal weight, with 23% of fetuses from LPD ($p=0.032$) and 28% from WD ($p=0.004$) fathers found above the 90th percentile for fetal weight. The fetal:placental ratios from LPD were also increased above the 90th percentile (25%; $p=0.015$). Targeted analysis of one-carbon metabolites revealed increased testicular methionine levels in MD-WD males compared to WD. Liver methionine, S-adenosylhomocysteine and S-adenosylmethionine was significantly increased in MD-WD compared to WD. Livers from WD males also demonstrated reduced levels of methionine, S-adenosylhomocysteine and homocysteine compared to LPD.

Conclusions: Paternal sub-optimal diets with and without methyl-donor supplementation altered the 1-carbon metabolomic profile of the testis and liver and disrupted the trajectories for fetal and placental growth. Further investigation of the impact diet has on the male reproductive physiology and the mechanisms by which this can influence fetal development are essential.

SHORT PAPER SESSION 1A: ACE PRE-REG SHORT PAPERS JEAN PURDY PRIZE

SP1A.1 Exploring the follicular steroid metabolome

Walker Jemma¹; Taylor Angela²; O'Reilly Michael²; Kirkman-Brown Jackson¹

¹Birmingham Women's & Children's NHS Foundation Trust; ²University of Birmingham, Institute of Metabolism and Systems Research

Purpose/background/objective: The Graafian follicle is an important steroidogenic unit made up of distinct cellular subtypes. Crosstalk between theca and granulosa cells has long been known to play an important role in ovarian production of steroid hormones. During the latter stages of follicular development, granulosa cells terminally differentiate into mural granulosa and cumulus cells. This study investigates the steroid metabolism of these cells in the human Graafian follicle.

Methods: Along with follicular fluid, mural granulosa and cumulus cells of patients undergoing IVF/ICSI treatment have been collected. Analysis of the steroid composition of the follicular fluid was undertaken using state-of-the-art mass spectrometry. Expression of steroidogenic enzymes within the cellular subtypes was assessed using real-time PCR. Further investigation of the functionality of these enzymes was undertaken through in vitro treatment of the cells with steroid precursors and assessment of the resultant metabolome by mass spectrometry. Comparisons of the enzymatic profile and metabolome between the two cell types and statistical analysis by paired t-test was performed. Subset analysis was undertaken to compare the function of the two cellular subtypes in patients with or without PCOS.

Results: Follicular fluid from IVF/ICSI patients contains high levels of pregnenolone, progesterone and 17OHP. The cumulus and mural granulosa cells express different steroidogenic enzyme signatures, particularly with HSD3B2 and CYP17A1. The two cell types also display different metabolite production profiles. Follicular fluid from PCOS and non-PCOS patients show differences in the metabolome of the Graafian follicle in these patient subgroups.

Conclusions: The Graafian follicle environment contains a range of steroids. The granulosa cell subtypes of the follicle have different steroidogenic potential and are able to contribute to the metabolome of the follicular environment. In future, assessment testing of women with impaired fertility.

SP1A.2 Ex vivo oocyte retrieval following controlled ovarian hyperstimulation at the time of bilateral salpingo-oophorectomy

Cohen Amy¹; Jansa Perez Marta¹; Lavery Stuart¹; Rattos Annabel¹; Butler Jon²

¹Wolfson Fertility Centre; ²The Royal Marsden

Background: A 20 year old woman diagnosed with serous ovarian tumours following laparoscopic investigation and biopsy was referred to the Wolfson Fertility Clinic with a view to performing ex vivo oocyte retrieval (OR) at the time of bilateral salpingo-oophorectomy. This procedure is only performed when routine in vivo egg collection risks dislodging tumour cells which could lead to spread of the disease. This case report is one of the few cases reported in the literature.

Methods: The patient was seen in clinic and a plan was made for her to undergo controlled ovarian hyperstimulation (COH). She attended the clinic for monitoring during COH. Close communication with the oncology team was maintained and surgery was scheduled. In the laboratory, a specific standard operating procedure was designed for the off-site ex vivo OR. On the day of OR, follicular fluid aspirated from the excised ovaries was checked. Cumulus-oocyte complexes (COCs) were identified and stored in HEPES buffered solution in a heated incubator. Following the procedure, gametes were promptly transported back to Wolfson Fertility Laboratory, and transferred into pre-equilibrated fertilisation media. The COCs were denuded and assessed for maturity. All mature eggs were vitrified.

Results: 13 COCs were retrieved, of which 10 oocytes were mature and have been cryopreserved. Histology performed on the ovaries confirmed findings of the previous biopsy. Test results show that patient's tumour marker levels continue to fall following chemotherapy. The patient will remain under close monitoring by the cancer treatment centre.

Conclusions: Fertility preservation is an important consideration when treatment for disease can result in infertility and may provide a viable treatment option for patients with limited options. Close liaison between multiple specialist teams is imperative to a successful outcome. We have since performed one more ex vivo OR.

SP1A.3 An analysis of lifestyle factors which may influence sperm maturity levels

Fryer Hayley; Drinkwater Abbie; Naik Rahul; Richardson Lucy

Herts and Essex Fertility Centre

Background: Mature sperm bind to hyaluronan in cumulus cells to initiate fertilisation. Immature sperm lack the receptors required to facilitate this binding and thus are unable to fertilise an oocyte. A hyaluronan-binding assay (HBA) can be used to assess sperm maturity and indicate whether physiological ICSI (PICSI) is required. This study aims to identify lifestyle and medical factors which may influence sperm maturity levels.

Methods: A retrospective analysis was completed on men attending semen analysis (SA) at Herts and Essex Fertility Centre between 07/03/2017 and 30/08/2019. Key SA parameters, lifestyle factors (age, BMI, smoking habits and alcohol consumption), and medical histories (testicular hydroceles, mumps, psychosexual issues, STDs, testicular trauma, torsion or swelling, operations to the testis, respiratory conditions, cardiovascular issues or diabetes) were compared between men with normal ($\geq 65\%$) and abnormal ($< 65\%$) HBA scores. Unpaired samples T-Test and Pearson's Chi Squared tests analysed statistical differences. Significance was accepted at $P < 0.05$.

Results: Patients with low HBA scores were more likely to suffer from diabetes and cardiovascular issues ($P=0.012$ and $P=0.046$ respectively) and were more likely to have had an operation on the testis ($P=0.025$). Sperm concentration and motility was also significantly lower in the reduced HBA group ($P<0.0001$). No significant differences were found in the other SA, lifestyle or medical factors examined.

Conclusions: Previous research has drawn links between conditions such as diabetes and cardiovascular disease with reduced semen parameters (1) (2). The implications that these conditions have on sperm maturity have not been explored in detail. As the current study suggests a possible link between diabetes and cardiovascular disease with reduced sperm maturity levels, it may be beneficial for patients with these conditions undergoing IVF treatment to request a HBA to determine whether PCSI may be beneficial for them.

- (1) Bhattacharya SM, Ghosh M, Nandi N. Diabetes mellitus and abnormalities in semen analysis. *The Journal of Obstetrics and Gynaecology Research*. 2013. 40 (1). 167-171.
 (2) Eisenberg ML, Li S, Behr B, Reijo Pera R, Cullen MR. Relationship between semen production and medical comorbidity. *Fertility and Sterility*. 2015. 103 (1). 66-71.

SP1A.4 Blastocyst quality is compromised in embryos presenting longer duration of compaction and direct cleavage

Fadon Patricia; Breen Danielle; Taylor Jonathan; Abdo Dima

Thames Valley Fertility

Introduction: Compaction is the initial marker of the onset of genomic activation. Studies have suggested that compaction on day 3 may be associated with better outcomes while late compaction is associated with reduced blastocyst formation rates^(1,2). There is yet to be a study that correlates compaction duration with direct cleavage and blastocyst quality. Direct cleavage has been reported to lower the chance of blastocyst formation but outcomes may be comparable in embryos that reach the blastocyst stage^(3,4).

Methods: 677 blastocysts were analysed using a time lapse imaging system -EmbryoScope™. Direct cleavage during the first cellular cycle (DC1) and duration of compaction (beginning of compaction to beginning of blastulation) were correlated with blastocyst quality. Blastocysts were classified as good (3BB or greater according to Gardner grading scheme) or poor quality (3BC, 3CB or lesser). A P value of 0.01 was taken as statistically significant. Correlation was determined using Pearson correlation coefficient analysis.

Results: Among the 677 embryos, 426 were considered good quality and 251 poor quality. The average duration of compaction for good quality embryos was 18.78h. This was significantly shorter than that of poor quality embryos, showing an average compaction duration of 21.79h ($p<0.01$). DC1 was observed in 6.45% (6/157) of top quality blastocysts (3AA or greater) which was significantly higher in very poor quality blastocysts (3CC or lower) 35.48% (114/251) $p<0.01$. There was a negative correlation ($r=-0.03$; $p<0.01$) between DC1 and duration of compaction thus, embryos presenting DC1 were most likely to have a longer duration of compaction and develop into poorer quality blastocysts.

Conclusion: DC1 and longer duration of compaction are correlated in poor quality blastocysts, suggesting their potential use as predictive markers for blastocyst quality.

- (1) Le Cruguel S, Ferré-L'Hôtelier V, Morinière C, et al. Early compaction at day 3 may be a useful additional criterion for embryo transfer. *J Assist Reprod Genet*. 2013;30(5):683-690. doi:10.1007/s10815-013-9983-3.
 (2) Skiadas CC, Jackson KV, Racowsky C. Early compaction on day 3 may be associated with increased implantation potential. *Fertil Steril*. 2006;86(5):1386-1391.
 (3) Zhan Q, Ye Z, Clarke R, Rosenwaks Z, Zaninovic N. Direct Unequal Cleavages: Embryo Developmental Competence, Genetic Constitution and Clinical Outcome. *PLoS One*. 2016;11(12):e0166398. Published 2016 Dec 1. doi:10.1371/journal.pone.0166398.
 (4) Desai N, Ploskonka S, Goodman LR, Austin C, Goldberg J, Falcone T. Analysis of embryo morphokinetics, multinucleation and cleavage anomalies using continuous time-lapse monitoring in blastocyst transfer cycles. *Reprod Biol Endocrinol*. 2014;12:54. Published 2014 Jun 20. doi:10.1186/1477-7827-12-54

SP1A.5 Investigating the effects of open and closed vitrification devices on mitochondria in human oocytes

Howard Rachel; Brison Daniel; Adeniyi Tope

Manchester University NHS Foundation Trust, UK

Background: Oocyte cryopreservation is used to prevent and treat female infertility. Irreversible damage to oocyte cellular organelles such as mitochondria can occur as a direct effect of vitrification. The effects of open vitrification (OV) and closed vitrification (CV) on oocyte structure and cellular organelles are debated within the literature.

Objectives: To investigate the differences in mitochondria number and distribution in fresh, OV and CV oocytes.

Methods: Research consented (with NHS REC approval and under an HFEA research licence) surplus metaphase II sibling oocytes (n=122) were randomised to fresh (n=35), OV (CryoTop, Kitazato, Japan) (n=36) and CV (Rapid-i, Vitrolife, Sweden) (n=36) treatment cohorts. Oocytes were incubated in 300nM Mitotracker Deep Red at 37°C for 30 minutes prior to fluorescence microscopy. Negative controls included non-stained oocytes and a cohort treated with the uncoupling agent FCCP. Images were captured using ZenPro and blinded for ImageJ analysis. Mean mitochondria density and mitochondria patterns were measured for all treatment groups.

Results: Cryo-survival rates were similar between CV (19/36 [52.8%]) and OV (16/36, [44.4%]) cohorts (p=0.48). No significant difference in mean mitochondria density was found between fresh (25.8 [12.7-44.9], SD 10.2, n=15), OV (30.3 [16.2-44.8], SD 10.1, n=11) and CV cohorts (26.6 [13.3-64.6], SD 15.3, n=12) (p=0.617). Significant differences in mean mitochondria density were found between fresh, vitrified and FCCP (18.4 [12.2-27.3], SD 4.91, n=10) treated oocytes (p=0.008). No significant difference was found between sibling oocytes in each treatment group (p=0.177). Two distinct patterns were observed during image analysis (n=36); mitochondria rings (MR) and mitochondria clusters (MC). Fresh (n=10) and CV (n=10) oocytes displayed more MR than OV (n=6) and FCCP (n=0) oocytes. All treatment groups displayed MC (n=10).

Conclusion: This data supports the use of either vitrification method clinically. More research is necessary to optimise vitrification methods in terms of efficiency and safety.

SP1A.6 Obstetric outcomes following donor egg IVF

Halley Beth; Moreno Katy; Skull Jonathan; Jivraj Shehnaaz

Jessop Wing, Sheffield Teaching Hospital NHS Foundation Trust

Introduction: Recent studies⁽¹⁻³⁾ have shown a higher risk of late pregnancy complications in women undergoing donor egg IVF (DE-IVF) with a wide variation in incidence. We sought to evaluate this risk in our own patient population in order to (i) better inform women undergoing DE-IVF of these risks (ii) compare our outcomes with published data. Outcomes assessed were, incidence of pre-eclampsia, mode of delivery, birth weight, gestational age at delivery, admission to the special care baby unit (SCBU).

Methods: Cases of DE-IVF were identified from the ACU database between 2000 and 2016. Pregnancy outcome data was obtained of those women who had their antenatal care and delivery in Sheffield. Women who had their antenatal care and delivery elsewhere were excluded from the study. As far as possible, data for comparison, was obtained from the local obstetric population in Sheffield. Where such information was not available, this was obtained from large datasets referenced below. Where appropriate statistical significance was tested using the chi-square test.

Results: 84 patients underwent DE-IVF between 2000-2016 at Jessop Fertility. Obstetric data was available for 36 women. Comparisons with published DE-IVF data and the general obstetric control population are illustrated below:

Conclusion: Whilst donor egg IVF enables women to achieve pregnancy and motherhood, it is associated with an increased risk of pre-term birth (p<0.05), pre-eclampsia, birth weight <2.5Kg (p<0.05) and SCBU admission. Patients are at significantly higher risk of delivery via caesarean section (p<0.05). Women undergoing donor egg IVF need to be counselled appropriately prior to embarking upon treatment. Our obstetric complication rates are in keeping with published studies in the world literature. We now have data we can quote from our own hospital and reassure ourselves and our patients that our outcomes are in keeping with expected standards of best practice.

1. Pecks, U. Maass, N. Nuelen, J., Dtsch Arztebl Int, 2011, Jan; 108(3) : 23-31

2. Wiggins, DA & Main E (2005) Outcomes of pregnancies achieved by donor egg in vitro fertilisation - a comparison with standard in vitro fertilisation pregnancies, Am J Obstet Gynecol; 192 (6):2002-6

3. O. Salha V. Sharma T. Dada D. Nugent A.J. Rutherford A.J. Tomlinson S. Philips V. Allgar J.J. Walker The influence of donated gametes on the incidence of hypertensive disorders of pregnancy Human Reproduction, Volume 14, Issue 9, September 1999, Pages 2268–2273.

SHORT PAPER SESSION 1B: BFS YOUNG CLINICIAN SHORT PAPERS

SP1B.1 Reproductive and perinatal outcomes using cryopreserved oocytes: an analysis of the HFEA database from 2010 to 2016 using three clinical models

Mascarenhas Mariano¹; Mehlawat Hazel²; Bhandari Harish M¹; Choudhary Meenakshi³

¹Leeds Fertility; ²Royal Grammar School, Newcastle upon Tyne; ³Newcastle Fertility Centre at Life

Background: How does the rising trend of oocyte cryopreservation for donor egg bank and social/fertility preservation indications impact live birth (LB) and perinatal outcomes.

Methods: We analysed 988 015 IVF cycles from the HFEA register from 2000 to 2016. Three clinical models were used to assess LB and perinatal outcomes: 1. Cryopreserved donor oocytes (n=922) vs fresh donor oocytes (n=24 706). 2. Cryopreserved autologous oocytes (n=632) vs cryopreserved donor oocytes (n=922). 3. First cycle of cryopreserved donor oocytes (n=917) vs first cycle of cryopreserved embryos using autologous oocytes (n=326). Singleton birth data was used for calculating perinatal outcomes. Preterm birth (PTB) was defined as LB before 37 weeks and low birth weight (LBW) was defined as birth weight <2500 gm. 100, 245 and 6537 singleton births were reported following cycles using autologous cryopreserved oocytes, cryopreserved donor oocytes, and fresh donor oocytes respectively.

Results: Model 1: The LB rate was lower in the cryopreserved donor oocyte arm than fresh donor oocyte arm (30.7% vs 34.7%, OR 0.835, 95%CI 0.724 to 0.962) Model 2: The LB rate was lower in the autologous cryopreserved oocyte arm than the cryopreserved donor oocyte arm (18.0% vs 30.7%, OR 0.497, 95%CI 0.388 to 0.636) PTB and LBW rates were not different in models 1 or 2. Model 3: The LB rate was lower following the first cycle of cryopreserved donor oocytes than the first cycle of cryopreserved embryos using autologous oocytes (19.3% vs 30.1%, OR 0.556, 95%CI 0.417 to 0.742). Whilst the PTB rate was not significantly different, the LBW rates were higher in the frozen donor oocyte arm (17.5% (14/80) vs 5.9% (9/152), OR 0.297, 95%CI 0.122 to 0.720)

Conclusions: These results indicate that women opting for cryopreserved oocytes should be informed regarding its impact on live birth rate and perinatal outcomes.

SP1B.2 Metabolic profiling to identify potential targets to improve the ovarian reserve

Al Rashid Karema¹; Taylor Amy²; Lawlor Debbie²; Nelson Scott¹

¹University of Glasgow; ²University of Bristol

Background: Interventions to improve the ovarian reserve are limited. We sought to identify whether specific metabolites which may be amenable to lifestyle interventions or supplementation were associated with the ovarian reserve biomarkers; anti-müllerian hormone (AMH) and antral follicle count (AFC).

Methods: Prospective cohort study of 399 infertile women intending to undergo IVF, with early follicular anti-müllerian hormone (AMH) and antral follicle count (AFC). Serum lipids, lipoprotein subclasses, and low-molecular weight metabolites were quantified by NMR spectroscopy (227 metabolic measures). Associations were analysed using regression models with adjustment for confounders.

Results: Participants were 35.5 ± 4.43 years, and had a median AMH of 16 pmol/l (IQR 8.8, 28.0 pmol/l) and median AFC of 12 (IQR 7,16). Neither AMH or AFC showed any association with an extensive lipid profile, while both biomarkers showed a positive association with the amino acid alanine; 0.11 (95%CI: 0.02,0.20) and 0.17 (95%CI: 0.08, 0.27) increase in one SD of AMH and AFC respectively per one SD increment in alanine. AMH also showed positive associations with total fatty acids, omega-3 fatty acids, omega-6 fatty acids and saturated fatty acids and additional associations with the amino acids; isoleucine, leucine and tyrosine, with effects ranging from 0.09 SD (95%CI: 0.002, 0.18) to 0.14SD (95% CI: 0.05, 0.24) increase in one SD of AMH per one SD increment in metabolite. There was also a negative association between acetate and AMH: -0.11 SD, (95%CI: -0.02, -0.20).

Conclusions: We did not identify any robust associations between AMH or AFC and an extensive lipid profile. Clarification of whether the observed positive associations between both fatty acids and some amino acids and markers of the functional ovarian reserve is causal is critical, given the potential for modification of these metabolites by dietary and supplement interventions.

SP1B.3 Agonist trigger does not confer a disadvantage in cycles where supraphysiological oestradiol levels are reached

Farahani Linda; Mittal Monica; Webber Lisa; Rai Raj; Salim Rehan

Imperial College Healthcare NHS Trust

Background: Oestradiol (E2) levels are routinely measured during an in-vitro fertilisation (IVF) cycle. When supraphysiological E2 levels are reached (>15000pmol/l) cycles are often converted to freeze-all cycles to reduce the risk of ovarian hyperstimulation syndrome (OHSS)¹. This study was conducted to ascertain whether GnRH agonist triggers(A) in these cycles were safe and effective.

Methods: A retrospective study of 4574 consecutive IVF/ICSI cycles conducted between 2014-2018.

Results: Data was collected from 4574 cycles. An hCG trigger (H) was utilised in 3070 cycles, and A in 399. Supraphysiological E2 levels were observed in 189 cycles. 111 cycles were triggered with H and 78 with A. There was no significant difference in age between the two groups (33.4 versus 34.0, p=0.35). There was a significant difference in antral follicle count (n=9.6(H) versus 12.7(A), p=0.03), oocyte number (n=18.59(H) versus 22.36 (A), p=0.0022), but no significant difference in day of transfer (p=0.7194) or quality of embryo transferred (p=0.2538). There was no significant difference in live birth rate (LBR) (30.4%(H) versus 24.7%(A), p=0.39). The use of hcg rescue in A cycles did not affect outcome. There was a significant difference in rates of OHSS between the two groups (27% (H) versus 9% (A)) when the peak E2 > 15000. Pregnancy outcomes in agonist trigger cycles at varying peak E2 levels were compared (<5000, 5000-10000, 10000-15000 and >15000pmol/l). There were no differences in LBR when A was used at any of these E2 levels (p<0.05). In H cycles, there was a significantly higher LBR in >15000 compared to <5000 cycles.

Conclusions: These results indicate that GnRH agonist triggers are not inferior and specifically in a cohort of patients where supraphysiological levels of E2 are reached, cycles may be safely and effectively managed with an agonist trigger, reducing the proportion of cancelled fresh transfers. These results can reassure both clinicians and patients that in fresh transfer cycles where supraphysiological oestradiol levels are reached, pregnancy outcomes are not negatively affected.

1. Blumenfeld Z. Why more is less and less is more when it comes to ovarian stimulation. J Assist Reprod Genet. 2015 Dec;32(12):1713-9. doi: 10.1007/s10815-015-0599-7. Epub 2015 Oct 19. PubMed PMID: 26481501; PubMed Central PMCID: PMC4681727.

SP1B.4 Kisspeptin and neurokinin B interactions in modulating gonadotropin secretion in women with PCOS

Skorupskaitė Karolina¹; George Jyothis T²; Veldhuis Johannes D³; Millar Robert P⁴; Anderson Richard A¹

¹MRC Centre for Reproductive Health, The Queen's Medical Research Institute, University of Edinburgh; ²Warwick Medical School, Coventry, UK; ³Endocrine Research Unit, Center for Translational Science Activities, Mayo Clinic; ⁴Centre for Neuroendocrinology and Mammal Research Institute, University of Pretoria, South Africa

Background: Polycystic ovary syndrome (PCOS) is characterised by abnormal GnRH/LH secretion. Neurokinin B (NKB) and kisspeptin are master regulators of GnRH/LH release, but their role in PCOS is unclear. We have investigated NKB and its interaction with kisspeptin in the regulation of the hypothalamic-pituitary-gonadal axis in women with PCOS using a specific neurokinin-3 receptor antagonist (NK3Ra).

Methods: Women with PCOS (based on the Rotterdam criteria) were either administered NK3Ra (MLE4901) 40mg bd for 7 days on day 1-2 of induced menstruation (n=8) or received no treatment, acting as controls (n=7). On the last day of NK3Ra dosing in the treatment group and the equivalent cycle day in controls, volunteers were randomised to receive a continuous intravenous infusion of kisspeptin-10 (4µg/kg/hour) or vehicle for 7 hours. This was repeated with the alternate infusion in a subsequent cycle. Plasma gonadotropin and estradiol were measured pre and post NK3Ra administration in the treatment group, including frequent sampling during 7 hour kisspeptin-10 or vehicle infusion for analysis of pulsatile LH secretion.

Results: NK3R antagonism reduced LH secretion (4.0 ± 0.4 vs 6.5 ± 0.8 IU/l, $p < 0.05$) and pulsatility (0.5 ± 0.1 vs 0.7 ± 0.1 pulses/hour, $p < 0.05$); FSH secretion was also reduced (2.0 ± 0.3 vs 2.5 ± 0.4 IU/l, $p < 0.05$). In PCOS controls, kisspeptin-10 increased LH secretion (5.2 ± 0.5 to 7.8 ± 1.0 IU/L, $p < 0.05$), with a positive relationship to estradiol concentrations. During NK3RA administration, the LH response to kisspeptin-10 was preserved (vehicle 3.5 ± 0.3 vs 9.0 ± 2.2 IU/l with kisspeptin-10, $p < 0.05$), but the positive correlation with circulating estradiol concentration was abolished ($r^2 = 0.59$, $p < 0.05$ in control cycles, $r^2 = 0.07$, ns after NK3R antagonist).

Conclusions: These data demonstrate the interactive regulation of GnRH/LH secretion by NKB and kisspeptin in PCOS, and that the NKB system mediates aspects of estrogenic feedback.

SP1B.5 Effect of transfer of a poor quality embryo along with a top quality embryo on the outcome during fresh and frozen in vitro fertilization cycles

Dobson Sam¹; Jayaprakasan Kanna²

¹Jessop Wing; ²Derby Teaching Hospitals NHS Trust

Objective: To evaluate the impact of a poor quality embryo (PQE) during double ET (DET) with a top quality embryo (TQE) on IVF outcome. Design: A review of prospectively collected data.

Setting: Tertiary level fertility clinic. Patient(s): All patients undergoing blastocyst transfers as part of fresh IVF ($n = 939$) and frozen ET ($n = 1,009$) cycles performed between 2010 and 2016.

Intervention(s): Single ET (SET) with TQE (group 1) was set as control and compared with outcomes for SET with PQE (group 2), DET with 2 TQEs (group 3), PQE plus TQE (group 4), and 2 PQE (group 5).

Main Outcome Measure(s): Live births and multiple births. Result(s): The live birth rates for group 4 were statistically similar to group 1 during fresh IVF (26.5% vs. 33.7%; odds ratio [OR], 0.95; 95% confidence interval [CI] 0.53--1.7) and frozen ET (24.2% vs. 32.7%; OR, 0.75; 95% CI 0.48--1.2), although there was a trend for lower success. Conversely, multiple births were higher in group 4 for fresh IVF (19% vs. 4.7%; OR, 2.9; 95% CI 1.3--6.6) and frozen ET (10.3% vs. 2.6%; OR, 2.4; 95% CI 1.2--4.9). The live birth rates for group 2 (12.2% for fresh IVF and 14.6% for frozen ET) and group 5 (21.2% for fresh IVF and 14% for frozen ET) were lower and for group 3 were higher (40.8% for fresh IVF and 40.3% for frozen ET) when compared with group 1. Multiple births were significantly higher with DET.

Conclusion(s): This study does not support DET with one PQE along with a TQE, when there is only one TQE and one or more PQEs available for fresh IVF or frozen ET.

SP1B.6 ICSI does not increase the odds of adverse perinatal outcomes in the absence of male factor subfertility: analysis of 131,686 singleton live births

Supramaniam Prasanna Raj¹; Lim Lee Nai¹; Granne Ingrid¹; Ohuma Eric²; McVeigh Enda³; Venkatakrishnan Radha⁴; Becker Christian³; Mittal Monica⁵

¹Oxford University Hospitals NHS Foundation Trust; ²Centre for Tropical Medicine and Global Health, Nuffield Department of Medicine, University of Oxford; ³Oxford Endometriosis CaRe Centre, Nuffield Department of Women's and Reproductive Health, University of Oxford; ⁴University Hospitals of Derby and Burton NHS Foundation Trust; ⁵Imperial College Healthcare NHS Trust, St Mary's and Hammersmith Hospitals

Background: An increased incidence of obstetric complications is recorded in pregnancies conceived through assisted reproductive treatments (ART) compared to spontaneous conception. Limited information is available on the effect of method of fertilisation, on perinatal outcomes in the absence of male factor subfertility.

Methods: All cycles recorded on the anonymised Human Fertilisation and Embryology Authority database between 1991-2016 were analysed retrospectively. All fresh cycles with normal sperm parameters resulting in a transferred embryo and singleton live birth were included. Frozen cycles, donor cycles, intrauterine insemination cycles and cycles where preimplantation genetic testing was undertaken were excluded. A total of 1,376,454 ART cycles were identified, of which 131,686 stimulated fresh cycle fulfilled the inclusion and exclusion criteria. Of these, 66,224 (50.3%) were IVF cycles and 65,462 (49.7%) ICSI cycles. Multivariate logistic regression was performed and a wider than normal

confidence interval of 99.5% was used to avoid clustering of cycles due to inability to link cycles¹. Statistical significance: $p < 0.005$ using Logistic Regression.

Results: IVF increased the risk of very preterm births (<32 weeks') (adjusted odds ratio [aOR] 1.25, 99.5% confidence interval [CI] 1.10-1.42, $p < 0.0001$) and preterm births (32-36 weeks') (aOR 1.10, 99.5% CI 1.03-1.17, $p < 0.0001$) compared to ICSI treatment cycles. The odds of having a post-dates delivery (>40 weeks') was reduced in IVF cycles (aOR 0.94, 99.5% CI 0.90-0.98, $p < 0.0001$) compared to ICSI. In contrast, a 7% (aOR 1.07, 99.5% CI 1.01-1.14, $p = 0.002$) higher proportion of large birth weight (LBW) (birthweight >4000g) babies were born following ICSI compared to IVF.

Conclusions: An exponential increase is seen in the number of ICSI cycles worldwide. This is the largest retrospective study to date evaluating the impact of method of fertilisation on perinatal outcomes, in the absence of male factor subfertility. ICSI is not associated with poorer perinatal outcomes in this cohort. IVF demonstrated 25% higher odds of having very preterm birth and 10% higher risk of preterm birth compared to ICSI. In contrast, IVF had an 11% and 6% lower chance of resulting in a term or post-dates delivery compared to ICSI. ICSI, however, was associated with a 7% higher chance of having a LBW baby compared to IVF.

1Sunkara Kamal, S., Khalaf, Y., Maheshwari, A., Seed, P., & Coomarasamy, A. (2014). Association between response to ovarian stimulation and miscarriage following IVF: an analysis of 124 351 IVF pregnancies. *Human Reproduction* (Oxford, England), 29(6), 1218

SHORT PAPER SESSION 1C: PSYCHOSOCIAL ASPECTS AND BFS NURSE PRIZE

SP1C.1 Analysis of 7810 consecutive counselling sessions: Deploying online technologies to improve access to care in a multi-location clinical setting

Sainsbury Tracey; Owen Janet; Coward-Evans Liz; McCann Mary; Ryb Anthony

London Women's Clinic

Background: The HFEA and BICA continue to emphasise the importance of provision of psychological support to patients at licenced centres. Changing patient and donor needs requires novel approaches to provide access to support.

As a multi-faceted programme involving large numbers of patients and donors across several sites, we sought improvements by the introduction of more flexible working patterns combined with deployment of multimedia solutions.

Methods: A qualitative and quantitative review of 7,810 counselling appointments between 2013 and 2018 provided by 9 BICA counsellors. Study parameters included the nature, context and proportion of appointments and feedback received from both patients and staff. Three key themes identified: need for out of hours counselling, shorter wait for support and increase in appointments attended.

Results: The context of sessions varied between gamete donors (16%), recipients (61%) using own gametes (8%) or social freezing (4%) and the nature of the sessions was assessed as main focus on implications (78%) or therapeutic/supportive (22%).

To address the needs identified, a cross-group Patient Support Team with fertility coach/ support coordinator was introduced and increased use of Skype, Zoom, WhatsApp video and telephone. This permitted empty slots (including from late cancellations) to be offered across all locations. Leading to a 100% increase in active use of counsellor time and 8 fold increase in the availability of slots between 7.30am and 7.30pm. A closed, counsellor led, Facebook group has enabled patients to access virtual 'in-clinic' support, providing peer support, themed chats and virtual support groups.

Conclusions: Our review and implementation of new practices in patients support services coincided with the change in the HFEA Code of Practice. The results illustrate the benefits of deploying modern technologies and flexibility for the provision of support to patients and donors within multi location centre.

SP1C.2 Mental health aspects related to male infertility - are men being overlooked?

Martin Anastasia; Brunckhorst Oliver; Ahmed Kamran

Background: The impact of infertility on mental health is clear with an estimated 20% of infertile couples showing signs of anxiety and depression. Male factor infertility accounts for a third of infertility diagnoses in couples, but despite this, the focus of psychosocial research remains on the female partner. This review therefore aims to identify 1) the mental health burden in infertile men including risk factors 2) the impact of mental health problems on fertility parameters, 3) diagnostic tools currently available and 4) mental health management options currently utilised.

Methods: A broad literature search was conducted. Studies pertaining to mental health in infertile men accordance to our aims were selected for inclusion into the article.

Results: We identified the most common mental health traits in infertile men to be depression (incidence of 5.5% - 32.1%), followed by anxiety (between 3.7% and 60.6%). Low income, age and low spousal support increased the risk of developing such mental health traits. The mental health burden involved both a decreased quality of life as well as physical consequences including decreased sperm motility and sperm count. Diagnosis of mental health status is largely conducted via questionnaires such as the State Trait Anxiety Inventory (STAI) and the Beck's Depression Inventory. However, no standardised questionnaire specific for mental health in infertility are currently used. Once diagnosed, treatment options for infertile men are largely focused around counselling and education, but only 11.3 to 20% of patients received mental health support due to lack of awareness, access and embarrassment.

Conclusion: This overview of the literature highlights the prevalence, risk factors and physical consequences of mental health conditions in male infertility. Furthermore, findings highlight the need for the development of disease specific screening tools and a greater awareness of treatment options available for early diagnosis and effective treatment of those affected.

SP1C.3 Oestradiol concentrations at the time of triggering in Letrozole-IUI cycles are predictive of subsequent clinical pregnancy

Ambrose Pat¹; Thomas Riya²; Samet Rebecca¹; Roebuck Frances¹; Lyon Jennifer¹; Gaudoin Marco¹; Fleming Richard¹

¹Glasgow Centre for Reproductive Medicine Fertility; ²University of Glasgow

Purpose: Letrozole results more reliably in unifollicular development than Clomiphene. However, Letrozole is an aromatase inhibitor and the lower oestradiol levels may have an impact on endometrial development, which in turn might impact on implantation. We aimed to determine if oestradiol concentrations on the day of triggering could be predictive of subsequent outcome in Letrozole-IUI treatment cycles.

Methods: All IUIs were performed within 24 hours of the Ovitrelle injection in a single centre from January 2017 to July 2019 inclusive. Patients' age, BMI, AMH level, number of follicles > 10 mm diameter and oestradiol concentration on day of trigger were correlated against the positive pregnancy rate (PPR) and subsequent CPR. Patients were categorised within incremental 100 pmol/L oestradiol bands (< 300 pmol/L, 300-399 pmol/L, 400-499 pmol/L etc.).

Results: In women with oestradiol concentration < 300 pmol/L (N = 48) the PPR was 6% and the CPR was 6%. In the 300-399 pmol/L group (N = 27) the PPR was 7% (N = 2) and the CPR, 4% (N = 1). In the 400-499 pmol/L group (N = 31) the PPR was 19% (N = 6) and the CPR, 16% (N = 5). Increasing incremental oestradiol bands did not confer further benefit. Comparing oestradiol < 400 pmol/L (N = 75, Gp-Low), and > 400 pmol/L (N = 153, Gp-High), there was no difference in BMI, AMH or number of follicles. Although the Gp-High patients were older (35.7 vs. 34.3 years, P=0.013), they had a higher CPR (14% vs. 4%, P=0.023).

Conclusion: Oestradiol concentration appears to be associated with subsequent CPR. Whilst we cannot influence the oestradiol concentration in Letrozole cycles, if the oestradiol is < 400 pmol, patients can be counselled accordingly and may elect not to proceed with IUI, especially if they are paying for treatment themselves.

SP1C.4 UK fertility clinics need to improve emotional support to patients undergoing IVF treatment

Beaumont Lucy; Jacques Celine; Barkaoui Samya; Noublanche Caroline; Kotrotsou Mara; Louzada Julio; Hickman Cristina

Apricity

Objective: The objective of this study was to find out from patients what was their experience of infertility and infertility treatment.

Methods: In May 2016, the survey was completed by 434 infertile UK participants between ages of 25 and 45: 58% women, 42% men; 90% were in a couple; 64% underwent previous fertility treatment (mostly IVF: 70%, and mostly private 59%); equally distributed between London, North, South, Midlands (24%,27%,26%,23%).

Results: Participants took 17 months on average before speaking to a doctor. Before starting treatment, 89% felt 'Apprehensive' and 88% felt 'Stressed'. 71% considered stopping treatment during treatment (78% men, 65% women) due to (in order) negative emotional impact (64%), too long and uncertain process (38%), physical toll (37%), financial reasons (35%), strain on relationship (31%) and professional life (7%). 67% of men felt "left aside, not considered during treatment". 75% considered that fertility treatment created tension in their couple (19% sometimes, 5% not really). 78% felt their couple was stronger after treatment. Participants compared their level of stress going through treatment with disease or illness (76%), losing a job (75%), relocating (75%), divorcing (68%) and losing a sibling or close friend (54%).

Conclusion: The results clearly suggest that there is a need for UK clinics to increase emotional support to patients undergoing IVF treatment. Meeting patients needs, requirements and expectations, and managing their emotional wellbeing during treatment (including the male partner) should be considered by fertility professionals at the same level of importance as succeeding in achieving a healthy live birth.

SP1C.5 Enhancing the patient treatment experience and improving communication and adherence by means of a digital platform

Wright Kirsty-Lee¹; Kenny Charlie²; Ng Elin²; Steyn Francesca¹; Serhal Paul¹

¹The Centre for Reproductive and Genetic Health; ²SALVE

Research shows that better communication with our patients can enhance the treatment experience and contribute to a healthier outcome (RCN 2019). Fertility treatment can often be an overwhelming process for patients and they explain that they struggle to understand and fully comprehend the vast amounts of information provided by professionals often expressing concerns regarding adherence to ART medications and appointments. Fertility nurses in particular spend on average approximately 9-10 hours with patients every treatment cycle to explain the regime and pathway, providing ongoing information, advice and support throughout the process. This is often repeating information to ensure that the patient has had the time to both understand the advice and ask any further questions adhering to HFEA guidance on the provision of information. To enhance the patient experience and ensure that good and accurate information is communicated, a software application called Salve has been developed to support patients on their treatment journeys and provide a digital platform for healthcare professionals to communicate and liaise with patients. Salve provides a platform for the nursing team to update the treatment plan with next steps and instructions which are then displayed in the patients mobile app in real time. There is also an opportunity for patients to communicate directly with the nursing team via an instant messaging service reducing the need for patients to call the clinic. Information is automatically delivered to the patient freeing up nursing time which can be redirected to the provision of patient emotional support and wellbeing. This abstract provides an overview of how a nursing team have implemented the application and received training and support from the application team, to provide better means of communication between patients and healthcare professionals, to support adherence to treatment medication and appointments and to therefore provide a more positive patient experience.

1. The Royal College of Nursing, 2019. Principles of nursing practice. <https://www.rcn.org.uk/professional-development/principles-of-nursing-practice> [accessed 6th September 2019]

SHORT PAPER SESSION 1D: SRF PHD STUDENT PRIZE

SP1D.1 Oocytes mount a delayed DNA damage response involving APC-Cdh1 mediated proteolysis

Subramanian Goutham Narayanan; Greaney Jessica; Wei Zhe; Becherel Olivier; Lavin Martin; Homer Hayden

University of Queensland - Centre for Clinical Research

Background: DNA damage in somatic cells activates a G2-M checkpoint that inhibits Cdk1 via Chk1/Chk2-dependent phosphorylation^{1,2}. In contrast, DNA damaged G2-stage oocytes readily enter M-phase immediately following genotoxic drug treatment in vitro, pointing to a defective DNA damage response (DDR) in oocytes³. However, in vivo, most oocytes in the ovary are constitutively G2-arrested within follicles. One mediator of this G2-arrest in oocytes is APC-Cdh1-mediated proteolysis of the Cdk1 activator, cyclinB1⁴. It remains unknown how oocytes respond to DNA damage acquired in vivo and hence, which has been present for an extended period. Senataxin (Setx) encodes a DNA helicase required for maintaining genomic integrity^{5,6}. Consequently, Setx-deficient mitotic cells exhibit increased DNA damage when exposed to oxidative stress⁵. The role of Setx has not yet been studied in oocytes.

Methods: We studied an in vivo model of DNA damage in oocytes involving Setx knockout (Setx^{-/-}) mice. We quantified DNA damage using γH2AX immunostaining; analysed APC-Cdh1-mediated proteolysis in live mouse oocytes using time-lapse imaging and quantified levels of inhibitory-phosphorylated Cdk1 and activated Chk1/Chk2 kinases using Western blotting.

Results: We find that Setx^{-/-} oocytes prematurely accumulate DNA damage in vivo during ageing and surprisingly, undergo a G2-arrest. This G2-arrest can be replicated in young Setx^{-/-} oocytes via extended in vitro culture that increases both DNA damage and oxidative stress. Significantly, co-culture with anti-oxidants in vitro blunts DNA damage and rescues G2-arrest in Setx^{-/-} oocytes showing that arrest is due to DNA damage rather than Setx loss. Unexpectedly, we find that G2-arrest is due to augmented APCCdh1-mediated cyclin B1 proteolysis and not due to canonical Cdk1 phosphoinhibition.

Conclusions: Here, by studying Setx mutant mice, we identify a novel DDR in oocytes. This DDR evolves slowly over several hours following the initial insult and involves APCCdh1-mediated proteolysis rather than the canonical Chk1/Chk2-Cdk1 inhibitory phosphorylation

1. Connell MJ, Raleigh JM, Verkade HM, Nurse P. Chk1 is a wee1 kinase in the G₂ DNA damage checkpoint inhibiting cdc2 by Y15 phosphorylation. The EMBO Journal. 1997;16(3):545. 2. Liu Q, Guntuku S, Cui X-S, Matsuoka S, Cortez D, Tamai K, et al. Chk1 is an essential kinase that is regulated by Atr and required for the G2/M DNA damage checkpoint. Genes & Development. 2000;14(12):1448-59. 3. Marangos P, Carroll J. Oocytes progress beyond prophase in the presence of DNA damage. Curr Biol. 2012;22(11):989-94. 4. Reis A, Chang H-Y, Levasseur M, Jones KT. APCCdh1 activity in mouse oocytes prevents entry into the first meiotic division. 2006;8:539. 5. Suraweera A, Becherel OJ, Chen P, Rundle N, Woods R, Nakamura J, et al. Senataxin, defective in ataxia oculomotor apraxia type 2, is involved in the defense against oxidative DNA damage. J Cell Biol. 2007;177(6):969-79. 6. Groh M, Albulescu LO, Cristini A, Gromak N. Senataxin: Genome Guardian at the Interface of Transcription and Neurodegeneration. Journal of Molecular Biology. 2017;429(21):3181-95.

SP1D.2 Metabolomic analysis of mouse embryos to assess the impact of culture using uterine cell-conditioned media in a microfluidic device

Mancini Vanessa¹; Mc Keegan Paul J.²; Sherrod Stacy D.³; Rutledge Alexandra C.³; Codreanu Simona G.³; Picton Helen M.¹; Pensabene Virginia¹

¹University of Leeds; ²Hull York Medical School; ³Center for Innovative Technology (CIT), Vanderbilt University

This study reports the use of a microfluidic culture system [1] to improve in vitro embryo culture techniques. We have recently reported altered gene expression in mouse blastocysts generated following culture in microfluidic devices in uterine cell conditioned media (CM) [2]. Here we present the effects of CM on embryo development and metabolomic profiles. The microfluidic device was fabricated using soft-lithography techniques and has previously been shown to produce equivalent numbers of blastocysts to conventional microdrop cultures [1]. To produce CM, mouse uterine epithelial cells (Creative Bioarray, USA) were cultured in KSOMaa (Merck Millipore, UK) for 24h. Groups of 10 murine zygotes (B6C3F1xB6D2F1 strain, EmbryoTech, USA) were cultured in either CM in microfluidic devices or control KSOMaa in devices. A total of 90 embryos were used in each experimental group (N=9 replicate cultures). Media samples (40μl) were collected after 5 days of culture and analysed by reverse phase liquid chromatography and untargeted mass spectrometry analysis (RPLC-MS) to explore differential metabolomics between experimental groups. Metabolomic data were reviewed using Progenesis Q1 and statistical analysis was performed using ANOVA. Blastocyst

rates were significantly ($p < 0.05$) higher in CM ($73.7 \pm 15.2\%$) versus controls ($59.3 \pm 18.6\%$). We observed significant ($p < 0.05$, fold change $> |2|$) up-regulation of 385 compounds and down-regulation of 236 compounds in CM versus controls. Of these, 353 were identified using in-house databases. Importantly, we found significant increases in metabolites involved in the metabolism pathways of purine, pyrimidine and 5 amino acids. This indicates an increase in protein and DNA synthesis which may drive to increased cell number. This work represents the first metabolomic investigation of embryos cultured in microfluidic devices. We identified differences in metabolite composition of spent media from CM group compared to control. Further investigation will focus on the impact of specific metabolic pathways identified in this study on embryo development and implantation potential.

[1] Colucci F., McKeegan P., Picton H., Pensabene V. (2018) Mouse embryo assay to evaluate polydimethylsiloxane (PDMS) embryo-toxicity. Conf. Proc. IEEE Eng Med Biol Soc. 2018 [2] Mancini V., Lu J., Colucci F., McKeegan P. J., Huntriss J. D., Picton H. M., Pensabene V. (2019) On-chip mouse embryo culture: evaluation of effects of uterine cells-conditioned media on embryo development and gene expression. Conf. Proc. Fertility 2019

SP1D.3 Methionine, one-carbon metabolism and bovine preimplantation embryo development

Clare Constance E; Tutt Desmond AR; Kwong Wing-Yee; Sinclair Kevin D

School of Biosciences, University of Nottingham, UK

Introduction: Deficiency of one-carbon metabolites during the periconceptional period can lead to epigenetic alterations in chromatin methylation affecting genes critical for early development [1]. Methionine concentrations in mammalian embryo culture media range from 0 to $>200\mu\text{M}$ between formulations. We hypothesise that altering concentrations of methionine, the precursor of the universal methyl donor, S-adenosylmethionine (SAM), will affect development and the epigenetic regulation of gene expression during bovine embryo culture with long-term health consequences. Here we report the effects of altered methionine concentrations on preimplantation embryo development.

Methods: Germinal-vesical oocytes were matured, fertilised and zygotes cultured to D8 in custom-made media containing 0, 10, 50, and $500\mu\text{M}$ methionine across 8 replicates. Morphological stage and grade of D7/8 blastocysts was assessed according to IETS [2]. Cell lineage allocation was determined by immunofluorescence for SOX2, NANOG and SOX17. Sex ratio was determined by RT-PCR. Proportions were analysed using logistic regression assuming binomial errors and count data assumed Poisson errors.

Results: There was no effect of methionine on proportion oocytes cleaved following insemination. The proportion D7 blastocysts of inseminated and cleaved increased ($P < 0.001$) with increasing methionine up to $50\mu\text{M}$, but decreased at $500\mu\text{M}$. Total cell number in D8 blastocysts increased ($P < 0.001$) with methionine up to $50\mu\text{M}$, but decreased at $500\mu\text{M}$ (70 ± 2.5 , 84 ± 1.6 , 89 ± 1.6 and 71 ± 4.0 for 0, 10, 50 and $500\mu\text{M}$, respectively). Proportion of inner cell mass (ICM; ~ 0.30) was unaffected by methionine. Proportion of Grade 1 blastocysts increased ($P < 0.05$) with methionine up to $50\mu\text{M}$, but decreased at $500\mu\text{M}$ (0.18 ± 0.100 , 0.49 ± 0.085 , 0.61 ± 0.080 and 0.45 ± 0.113). The proportion male embryos was statistically similar for each concentration (0.23 ± 0.095 , 0.56 ± 0.078 , 0.60 ± 0.078 and 0.50 ± 0.066).

Conclusions: Altered methionine concentrations (within physiological limits) affects preimplantation embryo development. Consequences for epigenetic gene regulation, based on DNA extracted from the immunodissected ICM and trophectoderm cell lineages, are under investigation. Funder: BBSRC (BB/K017810/1) and DTP.

1. Clare CE, Brassington AH, Kwong WY, Sinclair KD. One-Carbon Metabolism: Linking Nutritional Biochemistry to Epigenetic Programming of Long-Term Development. *Annu Rev Anim Biosci.* 2019; 7: 263-87

2. Bó G, Mapletoft RJ. Evaluation and classification of bovine embryos. *Anim. Reprod.* 2013; 10(3): 334-48

SP1D.4 The effect of age on oocyte energy metabolism and mitochondrial activity and copy number in sheep oocytes, cumulus cells and peripheral blood

Gnanaprabha Keerthi; McKeegan Paul; Berenyi Erika; Lu Jianping; Picton Helen

University of Leeds

Background: Advancing maternal age is a vital clinical predictor of oocyte competence, but the precise impact of age on oocyte maturation potential and energy metabolism remains unclear. This study investigates the relationship between

age and oocyte and cumulus cell mitochondrial activity and copy number and their correlation with peripheral blood mitochondria in a sheep model.

Methods: Ovaries and peripheral blood were obtained from 10 euthanized ewes aged 1, 3, 6, 8 and 9, (n=2/age group). Follicular fluid, cumulus cells and oocytes were harvested and tracked from 10±2 individual follicles/animal. Mitochondrial (Mt) activity (assessed by Brilliant Cresyl Blue staining), and MtDNA copy number (assessed by qPCR), were quantified in individual MII oocytes following in vitro maturation and supporting cumulus and blood. Oocyte glucose, pyruvate and lactate metabolism were measured using a spectrometer.

Results: A significant reduction in oocyte maturation potential with advancing age was noted ($p<0.05$) (MII rate: 1yr 89%, 3yrs 62%, 6yrs 64%, 8yrs 14%, 9yrs 5%). Oocyte pyruvate consumption increased significantly with age (9yrs: 70.12 ± 5.72 pmol/oocyte/hr vs 1yr: 28.46 ± 2.86 pmol/oocyte/hr; $p<0.001$). Lactate release declined (1yr: 4.15 ± 0.58 pmol/oocyte/hr vs 9yrs: 1.70 ± 0.24 pmol/oocyte/hr; $p<0.0001$). Oocyte glucose consumption was significantly higher ($p<0.001$) in ewes aged 3 yrs compared with either 1yr or 9 yr old animals. MtDNA copy number significantly declined with advancing age in oocytes (947473 ± 153297 vs 287371 ± 31959 ; $p<0.0001$), cumulus cells (450.7 ± 262.6 l vs 92.8 ± 26.8 ; $p<0.05$) and blood (332.5 ± 120.2 vs 49.4 ± 13.6 ; $p<0.01$) in ewes aged 1- 3 years vs 6-9 yrs, respectively. Oocyte Mt activity declined progressively with age (1yr 90%, 3yrs 62%, 6yrs 63%, 8yrs 40%, 9yrs 38%).

Conclusion: These results demonstrate a clear relationship between oocyte maturation potential, oocyte energy metabolism, and Mt activity and copy number and advancing age. This study raises the prospect of linking oocyte quality to peripheral blood markers

SP1D.5 Evolutionary conserved conceptus-derived proteins CAPG and P4HB elicit a transcriptional response in human & bovine endometrial cells that may facilitate implantation

Tinning Haidee¹; Wang Dapeng¹; Taylor Alysha¹; Pullinger Anna¹; Sutton Ruth¹; Oikonomou Georgious²; Velazquez Miguel³; Thompson Paul³; Treuman Achim³; O'Connell Mary⁴; Forde Niamh¹

¹University of Leeds; ²University of Liverpool; ³University of Newcastle; ⁴University of Nottingham

Background: We tested the hypothesis that novel proteins produced by the bovine conceptus during the peri-implantation period of pregnancy are highly conserved across placental mammals, and modify the endometrial transcriptome to establish uterine receptivity to implantation in cattle and humans.

Methods: To test this hypothesis, we determined the level of sequence identity of conceptus-derived proteins CAPG and P4HB across placental mammals by constructing sequence identity matrices in Clustalw. To test if either protein elicited a transcriptional response in the endometrium of different species we produced recombinant bovine CAPG (rbCAPG) and P4HB (rbP4HB) in an E.coli expression vector system. Primary bovine endometrial epithelial cells (bEECs) were isolated from late-luteal phase uteri. Human immortalised Ishikawa endometrial epithelial cells (hEECs) and bEECs were treated for 24 hours with 1) Control, 2) Vehicle control, 3) rbCAPG (1µg/mL), 4) rbP4HB (1µg/mL) (n=3 biological replicates). Single ended 75bp RNA sequencing was performed and mapped to Ensembl genomes. Rsubread and DESeq2 was used to determine differentially expressed genes (DEGs), adjusted $p<0.05$. KEGG pathways were determined using WebGestalt.

Results: Sequence identity at the protein level between human and bovine CAPG and P4HB was 91% and 96% respectively. Treatment of epithelial cells with rbCAPG altered 537 DEGs in bovine but 0 DEGs in human cells, whereas rbP4HB treatment altered 444 DEGs in bovine and 42 DEGs in human. One transcript, meiosis specific nuclear structural 1 protein, decreased in expression in epithelial cells from both species. Overrepresented pathways associated with hEECs treated with P4HB included Hippo and cAMP signalling pathways, whereas Toll-like receptor, NFκB, NOD-like receptor and TNF signalling were enriched in bEECs.

Conclusions: These data indicate that CAPG and P4HB are highly conserved across human and cow, and may have a common functional role in eliciting a transcriptional response in endometrial epithelial cells to facilitate uterine receptivity to implantation.

SP1D.6 Developmental programming of porcine muscle progenitor cell fate by foetal growth restriction (FGR)

Cortes Yennifer¹; Stenhouse Claire²; Esteves Cristina¹; Ashworth Cheryl¹; Donadeu Xavier¹

¹The Roslin Institute; ²Texas A&M University

Background and Objectives: FGR is a significant cause of ill-health during adulthood, particularly in developing countries. FGR babies present reduced muscle mass at birth associated with a smaller number of fibres and a tendency to accumulate fat in muscle and other depots during growth. This phenotype significantly predisposes to metabolic and other diseases later in life. Being a litter bearing species, pigs display a relatively high incidence of FGR with at least one littermate being affected in most litters, and thus represent an excellent model to understand the effects of FGR on muscle development. Our aim is to understand developmental muscle progenitor cell programming by FGR.

Methods: Muscle samples were collected from fetuses of 5 litters during late gestation (day 90). FGR were defined as having a weight that was >2SD lower than the litter average. Mononuclear cell fractions were obtained from semitendinosus muscle and were differentiated under established myogenic or adipogenic conditions, followed by analyses of lineage markers by immunocytochemistry and PCR. In addition, paired muscle samples from FGR and normal fetuses were submitted for mRNA-sequencing.

Results: Cells from FGR fetuses had reduced myogenic capacity indicated by reduced fusion indices (14% vs 26%, $P<0.05$) and reduced expression of the skeletal muscle genes, MYH2 (110.0-fold) and MYH3 (4.9-fold). In contrast, cells from FGR fetuses had an increased ability to undergo adipogenic differentiation relative to normal fetuses resulting in increased expression of the adipogenic transcript, PPAR γ (79.3-fold, $P<0.05$). Finally, RNA-sequencing revealed changes ($FDR<0.1$) in a total of 64 genes between FGR and normal fetuses.

Conclusions: Porcine foetal progenitor cell populations in culture can replicate the FGR muscle phenotype, demonstrating developmental programming of these progenitor populations in-utero. FGR muscle displays significant transcriptional dysregulation including numerous metabolic and immune genes. This provide a powerful system to elucidate the mechanisms involved in the FGR.

SHORT PAPER SESSION 1E: IWAN LEWIS JONES PRIZE

SP1E.1 Identification of the most clinically effective method for seminal round cell differentiation, the diagnosis of leukocytospermia, and its relationship with semen quality

Androni Dimitra Apostolia; Harbottle Stephen; Harman Ruth; Ferraro Elisa; Grasso Matteo

Cambridge IVF, NHS Cambridge University Hospitals

Background: Semen analyses commonly include combined quantification of spermatogenic and non-spermatogenic round cells (RC) but without further differentiation. Non-spermatogenic RCs predominantly include peroxidase-positive white blood cells (WBC), and although excessive levels are associated with urogenital infection and inflammation, there is no clear consensus on their significance for male fertility. The World Health Organisation (WHO) define pathological leukocytospermia as $>1.0 \text{M WBC/ml}$ in the ejaculate and recommend peroxidase staining for RC differentiation at a cut-off value of $>1.0 \text{M RC/ml}^{(1)}$. Nevertheless, there has been marked controversy over the diagnostic value of these recommendations which, to date, have lacked systematic study⁽²⁾.

Aim: To evaluate the clinical validity of WHO-defined threshold values for leukocytospermia and deployment of peroxidase staining.

Methods: Semen samples were collected from 321 men between April and June 2019. Standard semen analysis was performed including peroxidase staining (LeucoScreenTM, FertiPro) according to WHO 2010 criteria.

Results: Leukocytospermia was observed in 15.6% (50/321) of patients, with a statistically significant reduction in median sperm concentration observed at the threshold of $>1.0 \text{M WBC/ml}$ (60.12 ± 4.02 vs 37.88 ± 13.2 , $p=0.03$). A statistically significant, weak negative correlation was observed between WBC count and sperm concentration ($r=0.0$).

122, $p < 0.05$). Concentrations of 1.2M RC/ml and 6.8M RC/ml were calculated as the 5th and 95th centile of the leukocytospermic population respectively.

Conclusion: Although a statistically significant reduction in sperm concentration was observed when the WHO leukocytospermia threshold was reached, further data collection and analysis is needed to add statistical power. Leukocytospermia screening at a threshold of ≥ 1.2 M RC/ml would be associated with a 5% risk of misdiagnosis, which is arguably of clinical significance when the limitations and methodological uncertainty of manual semen analysis and peroxidase staining are taken into consideration. As such, we recommend a clinical cut-off value of ≥ 1.0 M RC/ml.

1. WHO (2010). WHO Laboratory Manual for the Examination and Processing of Human Semen. 5th Edition.

2. Sandoval, J., Raburn, D. and Muasher, S. (2013). Leukocytospermia: Overview of diagnosis, implications, and management of a controversial finding. Middle East Fertil. Soc. J, 18: 129-134.

SP1E.2 Novel causes of 46XY DSD - The role of DHX37 in human fetal testis development

Deligiannis Spyridon Panagiotis¹; Matilionyte Gabriele¹; Tharmalingam Melissa Durgahshree¹; Rimmer Michael¹; Ferguson Linda¹; Brown Pamela¹; de Mendonça Berenice Bilharinho²; Mitchell Rod¹

¹University of Edinburgh; ²University of Sao Paulo

Background: Embryonic testicular regression syndrome (ETRS) represents a 46XY Disorder of sex development (DSD) with variable phenotype. However, the underlying molecular mechanisms causing ETRS remain mostly unknown. Recently, the RNA helicase, DEAH (Asp-Glu-Ala-His) box polypeptide 37 (DHX37), has been postulated to be involved in cases of DSD with male-to-female sex reversal. This study aimed to characterise DHX37 and investigate the consequences of DHX37 repression in human fetal testis.

Methods: To localise DHX37 expression, immunofluorescence was performed for DHX37, Sertoli cells (SOX9), gonocytes (AP2γ), pre-spermatogonia (MAGE-A4) and Leydig cells (CYP11A1) using first (n=4, 10-12GW) and second (n=8, 13-22GW) trimester human fetal testis tissue. For genetic manipulation, a Lentiviral-delivered miRNA system was used. Validation was performed in NT2 (embryonal carcinoma) cell line with qPCR analysis of DHX37 mRNA levels. For hanging drop cultures, second trimester (n=3, 16-20GW) human fetal testes tissue pieces were exposed to DHX37 miRNA (miR232, miR280 or miR423) or scrambled control for 24hrs. Tissue was transferred to fresh media and cultured until day 14. Immunofluorescence was performed for DHX37 and SOX9 for cell quantification. Data were analysed by Two-way ANOVA.

Results: DHX37 is expressed in most of the cell types (Sertoli cells, gonocytes, pre-spermatogonia, Leydig cells) in human fetal testis. Lentiviral transduction in NT2 cells resulted in >70% reduction in DHX37 mRNA expression with all three miRNAs compared to control. Significantly lower number of cells expressing DHX37 was observed in tissue treated with miR280 and miR423 ($p < 0.05$). Moreover, a significant reduction (approximately 40%) in Sertoli cell number was observed in tissue treated with miR232 ($p < 0.001$), miR280 ($p < 0.01$) and miR423 ($p < 0.05$) compared to control.

Conclusions: Repression of DHX37 leads to a reduction in Sertoli cell number in human fetal testis which is likely to impact on subsequent gonadal development. The long-term effects of DHX37 repression are currently under investigation.

SP1E.3 HABSelect: Relationships between measures of sperm DNA integrity, baseline semen sample data and the trial's clinical outcomes

Miller David¹; Partanen Riitta¹; Hargreaves Alex¹; Torabi Forough¹; Frew Lorraine²; Tsagdi Sofia²; Hutton Rachel³; Romana-Montanara Claudia²; West Robert¹; Kirkman-Brown Jackson²; Lewis Sheena³

¹University of Leeds; ²University of Birmingham; ³Queen's University Belfast

Introduction: The HABSelect randomised clinical trial was designed to test a hyaluronan-based sperm selection platform for treating male infertility. Part of our remit was to mechanistically relate the trial's clinical outcomes to sperm hyaluronan binding scores (HBS), DNA integrity and other semen sample parameters (1). Herein, we report that sperm DNA integrity assays related to clinical outcomes and differing semen parameters including HBS but not uniformly so.

Methods: Couples (N=2,772) in 16 independent treatment centres were recruited.

Following randomisation (PICS or ICSI), clinical outcome and semen parameter data for 1,247 couples were available for analysis with multiple, slide-based assays of DNA integrity (HALO, Aniline Blue, Acridine Orange, Comet and TUNEL assays) obtained from residual, processed, frozen-stored samples. Associations were tested by contingency tables and logistic regression adjusted for female age with uninformative assays dropped by parsimonious modelling as appropriate.

Results: Clinical pregnancies (N=518; 8 lost to follow-up) led to 418 live births and 92 miscarriages. Sperm HALO area and aniline blue staining discriminated, respectively, between no pregnancy and biochemical pregnancy ($p=0.018$) and between biochemical and clinical pregnancy ($p=0.016$). Acridine orange and the Comet assays both discriminated between live birth and miscarriage ($p=0.033$). TUNEL was not discriminatory for any clinical outcomes. Sample sperm concentration/HBS and motility correlated, respectively, with DNA fragmentation measured by acridine orange ($P<0.0001$) and the Comet assay ($p<0.0001$).

Conclusions: Highly variable measures of DNA fragmentation were recorded and inter-assay correlations were weak among sperm samples. Assay measures correlated better with semen sample parameters than with clinical outcomes. Sample processing and freezing may have contributed to the poor performance of assays overall (2, 3) and freeze shock may have revealed nuances in sperm chromatin structure leading to the differential assay performance according to clinical outcome.

1. Kirkman-Brown J, Pavitt S, Khalaf Y, Lewis S, Hooper R, Bhattacharya S, et al. Sperm selection for assisted reproduction by prior hyaluronan binding: the HABSelect RCT. Efficacy and Mechanism Evaluation, Southampton (UK) 2019. 2. Le MT, Nguyen TTT, Nguyen TT, Nguyen TV, Nguyen TAT, Nguyen QHV, et al. Does conventional freezing affect sperm DNA fragmentation? Clinical and experimental reproductive medicine. 2019 Jun;46(2):67-75. PubMed PMID: 31181874. PubMed Central PMCID: 6572667. 3. Gomez-Torres MJ, Medrano L, Romero A, Fernandez-Colom PJ, Aizpurua J. Effectiveness of human spermatozoa biomarkers as indicators of structural damage during cryopreservation. Cryobiology. 2017 Oct;78:90-4. PubMed PMID: 28641947.

SP1E.4 The impact of vincristine treatment on prepubertal mouse testis

Allen Caroline; Parveen Aisha; Lopes Federica; Mitchell Rod; Spears Norah

University of Edinburgh

Infertility is a common side effect of cancer therapy during childhood. Gonadotoxicity and associated infertility risk of chemotherapy agents used in paediatric oncology is debated and often based on adult patients (1). Vincristine is a microtubule-destabilizing agent that disrupts cell division and is used to treat a number of cancers including acute lymphoblastic leukaemia, the commonest malignancy of childhood. This drug is considered 'low risk' for gonadotoxicity; however, the direct effects of vincristine have yet to be fully investigated for prepubertal testis (2). Here, we determined the impact of vincristine treatment on the germ cell population in prepubertal mouse testes. Prepubertal testes were collected from postnatal day five CD1 mice and fragments cultured for five days. Vincristine was added on the second day of culture for 24hrs at 0.001, 0.01, 0.1, 1 or 10 μ g/ml, concentrations in the range of published patient serum levels of 0.001-0.5 μ g/ml (3). Addition of BrdU for the final 24hrs of culture enabled assessment of proliferation. Density and proliferative capacity of the germ cell population was evaluated through manual counting of cells from immunofluorescent images. Vincristine treatment had a significant effect on the density of the germ cell population at 0.1, 1 and 10 μ g/ml ($p<0.01$). The germ cell population decreased by ~70% and accumulated within the centre of seminiferous tubules. These germ cells varied in their shape and size with expanded and shrunken morphology observed. Vincristine specifically reduced the density of the proliferating germ cell population by ~90% ($p<0.01$) whilst having no impact on the population of germ cells that were not undergoing proliferation. Overall, vincristine has a significant effect on the germ cell population at patient relevant concentrations based on this short-term animal model study. Further studies of long-term effects, as well as human tissue studies, are required to determine the clinical relevance of these results.

1. Wallace WHB, Anderson RA, Irvine DS. Fertility preservation for young patients with cancer: who is at risk and what can be offered? Lancet Oncol. 2005;6(4):209-18. 2. Allen CM, Lopes F, Mitchell RT, Spears N. How does chemotherapy treatment damage the prepubertal testis? Reproduction. 2018;R209-33. 3. Crom WR, de Graaf SS, Synold T, Uges DR, Bloemhof H, Rivera G, et al. Pharmacokinetics of vincristine in children and adolescents with acute lymphocytic leukemia. J Pediatr. 1994 Oct;125(4):642-9.

SP1E.5 The relationship between P4 and PGE1 induced intracellular calcium increase in human sperm and correlation to IVF outcome

Dean Morven¹; Brown Sean²; Martins da Silva Sarah¹; Barratt Christopher¹

¹University of Dundee; ²University of Abertay

Background: Evidence from genetic knock-out mouse and humans demonstrate that the sperm-specific channel, CatSper, is critical for regulation of intracellular calcium ([Ca²⁺]_i) and fertilisation competence. Additionally, suboptimal elevation of [Ca²⁺]_i by progesterone (P4) is associated with failed fertilisation. As P4 activates CatSper indirectly it is unclear if such responses are due to low channel expression or impaired upstream signalling. In contrast, prostaglandin E1 (PGE1) has been reported to activate the channel directly.

Aim: To investigate the relationship between P4 and PGE1 induced [Ca²⁺]_i influx in patient populations undergoing ART in comparison healthy donor. Furthermore, to correlate this with IVF success in patients.

Methods: Population and single-cell fluorometric [Ca²⁺]_i screening assays were used to examine the relationship between P4 and PGE1 in both study groups. P4/PGE1 [Ca²⁺]_i data are expressed as induced peak relative to maximal fluorescence induction with ionophore A23187 (ΔF Ca²⁺).

Results and Conclusions: Responses to P4 and PGE1 are positively correlated ($R^2 = 0.47$) in donor sperm population ($n=29$) and in the IVF patient population group ($R^2 = 0.68$, $n=61$). In single cell responses, mean P4 and PGE1 were not correlated in donors ($R^2=0.001$, $n=17$) or IVF patients ($R^2=0.01$, $n=31$). There was no relationship between P4 and PGE1 responses in both assays and fertilisation outcome although it is worth noting there were no totally failed fertilisation in this patient cohort. However, mean single cell P4 and PGE1 responses between donor and patient sperm are statistically different ($p<0.0001$) and impaired [Ca²⁺]_i signalling may contribute to patient subfertility. The cause and consequence for sperm function of the increased heterogeneity of agonist induced [Ca²⁺]_i, particular in vivo, remains to be determined.

Kelly, M.C., Brown, S.G., Costello, S.M., Ramalingam, M., Drew, E., Publicover, S.J., Barratt, C.L. and Martins Da Silva, S., 2018. Single-cell analysis of [Ca²⁺]_i signalling in sub-fertile men: characteristics and relation to fertilization outcome. *Human Reproduction*, 33(6), pp.1023-1033. Williams, H.L., Mansell, S., Alasmari, W., Brown, S.G., Wilson, S.M., Sutton, K.A., Miller, M.R., Lishko, P.V., Barratt, C.L., Publicover, S.J. and Martins da Silva, S., 2015. Specific loss of CatSper function is sufficient to compromise fertilizing capacity of human spermatozoa. *Human Reproduction*, 30(12), pp.2737-2746. Miller, M.R., Mannowetz, N., Iavarone, A.T., Safavi, R., Gracheva, E.O., Smith, J.F., Hill, R.Z., Bautista, D.M., Kirichok, Y. and Lishko, P.V., 2016. Unconventional endocannabinoid signaling governs sperm activation via the sex hormone progesterone. *Science*, 352(6285), pp.555-559.

SP1E.6 Recovery of spermatogonial stem cells after in vitro exposure to chemotherapy drugs in a prepubertal mouse model

Lopes Federica¹; Tholeti Prathima²; Adiga Satish K²; Mitchell Rod T¹; Spears Norah¹

¹University of Edinburgh; ²Manipal Academy of Higher Education

Background: Lifesaving chemotherapy treatment in childhood cancer patients can reduce male fertility during adulthood¹. Here, we studied the effect of cisplatin (CIS) and doxorubicin (DOX) commonly used in the treatment of oncologic paediatric patients, upon the immature testis shortly after drug exposure plus after the first and second round of spermatogenesis.

Methods: Testis from neonatal CD1 mice were fragmented into $\sim 1\text{mm}^3$ pieces and cultured in vitro in α MEM, 10%KSR and 1%antibiotic-antimycotic, at 34°C in 5%CO₂. Fragments were exposed for 24h (Day2) to human-relevant concentrations of CIS (0.25, 0.5 or 0.75 μgml^{-1}) or DOX (0.01, 0.03 or 0.05 μgml^{-1}), while control fragments treated with vehicle. On Day3, testis fragments were moved into drug-free medium. Twenty-four hours before histological processing ($n=5-14$), BrdU was added. Three time points were selected: week 1, 4 and 8. Immunohistochemistry was performed to identify PLZF+ (spermatogonial stem cells, SSCs), BrdU+ (proliferating cells), DDX4+ (germ cells) cell populations. Morphological analysis assessed the presence of round (RS) and elongated (ES) spermatids.

Results: One week after drug exposure, all concentrations of CIS and DOX (except lowest DOX dose) had dramatically reduced SSCs, germ cells and proliferating cells densities compared with control ($p<0.001$ for all). Nonetheless, 4 and 8 weeks after treatment, SSCs, germ cells and proliferating cells densities no longer differed from control (with the exception of CIS and DOX at the highest doses, $p<0.05$). Crucially, the proportion of proliferating SSCs did not change

over time and across treatments (20-50%). Notwithstanding such recovery, the percentage of testis fragments with RS at week 4 and ES at week 8 were reduced to at least half of that found in controls (54% for both).

Conclusion: Despite early loss of SSCs in prepubertal testis, spermatogenesis might partially recover after an extended period post-chemotherapy, although a reduction in mature germ cells may result in lower fertility.

1. Chow EJ, Stratton KL, Leisenring WM, Oeffinger KC, Sklar CA, Donaldson SS, et al. Pregnancy after chemotherapy in male and female survivors of childhood cancer treated between 1970 and 1999: A report from the Childhood Cancer Survivor Study cohort. *The Lancet Oncology*. 2016;17:567–576.

SHORT PAPER SESSION 2A: ACE POST REG

SP2A.1 Developing an IVF/ICSI success prediction test based on the vaginal microbiome

de Jonge Jonathan¹; Koedooder Rivka²; Schoenmakers Sam²; Macklon Nicholas³; Laven Joop²

¹ARTPred; ²Erasmus University Medical Centre; ³London Women's Clinic

The human microbiome in health and disease is increasingly appreciated due to recent development of culture-independent sequencing techniques such as next generation sequencing (NGS) and interspace region based platforms (ISPro). Associations between presence of pathogens in the lower female reproductive tract and adverse pregnancy outcomes after both natural and in vitro fertilization (IVF)/intracytoplasmic sperm injection (ICSI) conceptions have been reported as well as the role of the entire vaginal microbiome in fertility. In a Dutch multi-centre study (Koedooder 2019), the vaginal microbiome profiles of 192 women were determined using IS-Pro and NGS in vaginal and urine microbiomes before the initiation of the IVF/ICSI-procedure. The profiles were shown to be predictive of treatment outcome, and implantation failure was associated with distinct features of the vaginal microbiome. The ISPro-technique offered a simpler, faster and cheaper alternative to NGS, and the technique was employed to develop a predictive IVF-assay named ReceptIVFity. ReceptIVFity predicts the chances for successful IVF/ICSI with an embryo-transfer up to two months after sampling by stratifying vaginal microbiome profiles into three groups (successrate pregnancy): LOW (5.9%), MEDIUM (23.8%) and HIGH (52.6%). Prediction was optimized for robustly predicting LOW (specificity 97%, predictive accuracy of 94%). An external validation set in a German IVF-center (50 patients included) revealed a 100% predictive accuracy (Koedooder 2019). ReceptIVFity (ARTPred, Den Bosch, NL) is now CE-certified for Europe and used by clinics in the Netherlands, Germany, Denmark and the United Kingdom. A leading Dutch health-insurer has reimbursed ReceptIVFity as the new standard of care for all IVF/ICSI-patients in the two largest Dutch IVF-clinics. Novel analytic techniques that enable interrogation of the whole vaginal microbiome rather than the few bacteria implicated in bacterial vaginosis promise to offer new applications. The search is now on for therapeutic interventions that can improve the microbiome, and with it clinical outcomes.

SP2A.2 Volatile Organic Compounds: Does CE marking fail the smell test?

Jaques Sophie; Whitten Bryony; Gregoire Rachel

Hewitt Fertility Centre Knutsford, UK

Background: Disposable plastic consumables used in IVF have previously been shown to emit volatile organic compounds (VOCs)⁽¹⁾. Licence condition T30 of the Human Fertilisation and Embryology Authority (HFEA) 9th Code of Practice, specifies that CE marked products are used wherever possible⁽²⁾. It is unclear if VOC levels are measured when a product is deemed 'IVF safe' by CE marking.

Aim: To assess VOC levels in CE marked IVF consumables.

Methods: A VOC meter (miniRAE 2000, RI Ltd) was used to measure VOC levels in parts per million (ppm) in semen containers (ReproMed Semen Container [113101]; Oosafe Sperm Cup [OOPW-SC01]), 60mm petri dishes (Nunc™ 60mm petri dish [150270]; Vitrolife Culture Dish 60mm [16002]) and embryo transfer catheters (Wallace Classic Catheter [1816N]; Kitazato Catheter [223340]). Readings were taken after packaging and product opening; at 10 minute intervals for one hour; hourly intervals for 6 hours, at both room temperature (22°C) and 37°C. A mean was calculated from three different batches. VOC levels of ≥0.2ppm were considered high.

Results: High VOC levels were recorded in ReproMed Semen Containers (2.87ppm at 22°C; 12.9ppm at 37°C), Nunc™ 60mm dishes (3.53ppm at 22°C; 5.07ppm at 37°C) and Oosafe Sperm Cup (2.13ppm at 37°C). Oosafe Sperm Cups emitted significantly higher VOCs at 37°C compared to 22°C ($p=0.041$). After off-gassing to 0.0ppm, VOC levels of Oosafe Sperm Cups at 37°C (0.53ppm) and ReproMed Semen Containers at 22°C (0.43ppm) and 37°C (0.97ppm), remained high after 6 hours.

Conclusion: Clinics must independently VOC test all consumables as CE marked products deemed 'safe for IVF' emit dangerously high VOC levels. Consequently, the Hewitt Fertility Centres have reverted to using a non-CE marked semen container as a suitable, CE marked option is not currently available. We must encourage transparency from suppliers regarding VOC testing protocols as CE marking alone is insufficient.

1. Gilligan, T., Schimmel, T., Esposito, B., Cohen, J. 1997. Release of volatile organic compounds such as styrene by sterile petri dishes and flasks used for in-vitro fertilization. *Fertility and Sterility*. 68(s1),52-53
2. Human Fertilisation and Embryology Authority (2018a) Code of Practice 9th Edition, HFEA: London

SP2A.3 Cell fusion in the preimplantation human embryo is not associated with a significantly decreased live birth rate

Davis Natalie¹; Campbell Alison²; Smith Rachel³; Best Louise²; Duffy Samantha⁴; Montgomery Sue⁴; Wheat Stacy⁵; Davies Laina⁶; Foad Fiona⁷; O'Boyle Sarah⁸

¹CARE Fertility Northampton; ²CARE Fertility Group; ³CARE Fertility Sheffield; ⁴CARE Fertility Manchester; ⁵CARE Fertility London; ⁶CARE Fertility Nottingham; ⁷CARE Fertility Tunbridge Wells; ⁸Beacon CARE Fertility Dublin

Cell fusion, defined as a reduction in cell number during the cleavage stage due to cells merging is considered an aberrant embryo development pattern. This study aims to determine the effect of cell fusion on live birth outcome. 2612 embryos from 7 clinics sharing an annotation protocol, with known live birth data (KID) were cultured in the EmbryoScope™ (Vitrolife, Sweden) and transferred at the blastocyst stage. 'Merged cells' was prospectively annotated when a cell completed division and cell fusion then occurred. The live birth rates (LBR) of embryos exhibiting cell fusion (group 1) and those which did not exhibit cell fusion (group 2) were assessed. Significance was calculated using the chi squared test. The effect of cell fusion was then compared to the clinic morphokinetic model which categorises embryos into low (C), medium (B) and high (A) potential for live birth to observe their combined effect. Within the KID embryo cohort 1.8% (1dp, 47/2612) embryos underwent cell fusion. The LBR of groups 1 and 2 were 21.3% (10/47) and 29.2% (748/2565) respectively ($p=0.31$, Not significant (NS)). When assessed alongside the morphokinetic model, 87.2% (41/47) cell fusion events occurred in group C embryos. The cell fusion group LBR was 17.1% (7/41) vs 24.1% (274/1337) without cell fusion ($p=0.74$, NS). This study shows a non-significant trend towards lower live birth rate when cell fusion occurs, compared to when it does not. This may be due to the low number of embryos displaying cell fusion, as clinic policy excludes these from transfer if standard cleaved embryos are available. 87.2% embryos exhibiting cell fusion fall within morphokinetic model group C and have an equivalent live birth rate to this group, suggesting that they should continue to be selected against where embryos which class in groups A or B in our morphokinetic model are available.

SP2A.4 The Geri time-lapse incubator significantly improves multiple IVF laboratory outcomes compared to conventional benchtop incubation

Thomson Andrew¹; Palmer Giles¹; Sprober Peter¹; Meredith Melanie¹; Thackare Hemlata¹; Macklon Nick²; Ahuja Kamal²

¹London Women's Clinic Wales and Bristol; ²London Women's Clinic

An estimated 1.2 million IVF cycles have been performed worldwide using time-lapse incubators (TLI). The potential advantages of TLI include maintaining stable culture conditions and limiting the exposure of embryos to gas and temperature fluctuation. Multiple recent meta-analysis and randomised controlled trials have concluded that TLI improve pregnancy and livebirth rates as well as reducing early pregnancy loss (Pribenszky et al, 2017). Despite this, TLI is still "amber lighted" by the HFEA and not recommended for use by NICE guidelines. This retrospective study assesses outcomes of the Geri TLI vs traditional benchtop incubators (BTI) Method: Cycles performed at London Women's Clinic Wales between Jan 2015-Dec 2018 were analysed. Patients were assigned to Geri TLI or BTI at the discretion of the embryologists and space availability. Patients aged under 39, undergoing their first or second cycle with fresh ET were included. PGT, egg donation and surrogacy cycles were excluded. Patients were split into two groups for analysis: exclusive BTI (n=402) or exclusive TLI (n=232). Results: Rates of blastocyst formation (32.6 vs 65.6% $p<.05$), blastocyst embryo transfer (63.7 vs 80.2%, $p<.05$) and surplus blastocyst vitrification (19.1 vs 31.3%, $p<.05$) were significantly

higher for embryos cultured in TLI. Fertilisation (68.5 vs 67.9%) and implantation (34.3 vs 37.9%) rates were not significantly different. Miscarriage rate was similar (12% vs 8%). Patient demographics were similar; average patient age was 32.6 vs 32.7 years, with average 8.1 vs 8.9 eggs collected. Conclusion: This retrospective study provides four years of IVF laboratory data which favours TLI. Although implantation rate was not significantly different with fresh embryo transfer, the TLI group is likely to have a higher cumulative pregnancy rate from one egg collection event as blastocyst formation and vitrification rates were higher in Geri. Further prospective controlled studies are required for confirmation.

SP2A.5 Optimising FET outcome: the effect of post-warm blastocyst re-expansion and time to embryo transfer

King Rebecca; Thomas Victoria; Walker Rachel; Heath Felicity; Knaggs Paul

Wales Fertility Institute

Purpose: Currently there is no consensus about whether embryo warm to embryo transfer interval influences FET outcome. Previously we only used embryo survival as the sole factor to determine whether or not to transfer a particular embryo. Our aim was to investigate the time-to-transfer interval and its influence on post warm re-expansion status in an effort to understand their effects on outcome and also to inform our choices regarding embryo thaw strategy.

Methods: This was a retrospective study using data from 315 day five vitrified embryos from September 2017 to April 2019. The embryos were vitrified and warmed using GEMs or Cook media. Re-expansion data was collected one hour post-warm and during transfer.

Results: At one hour post-warm, fully re-expanded embryos had a CPR of 35.1% compared to 21.3% of partially re-expanded and 16% of collapsed embryos ($p=0.021$). When time-to-transfer was examined, the CPR was greater after 3-4hours (35.8%) and 4-5hours (31.6%) compared to 0-1hours (0%), 1-2hours (18%) and 2-3hours (25.2%) ($p=0.046$). When re-expansion status at transfer and time-to-transfer were looked at simultaneously, the highest CPR was in the fully expanded 3-4hour group with a CPR of 42.1%. This was supported by the CPRs at 2-3hours (32.9%) and 4-5hours (35.7%) for fully re-expanded embryos. Partially re-expanded embryo CPRs consistently stayed at about 16% despite the time in culture. Binomial Logistic Regression determined 10% of the variance in CPR could be explained by re-expansion and time-to-transfer ($p<0.0001$).

Conclusions: The best FET clinical pregnancy rates are achieved by culturing for three to five hours post-warm and transferring fully re-expanded blastocysts. Our protocol is now to culture thawed embryos for a minimum of three hours. We also consider warming a second embryo for transfer if none or little expansion is seen within an hour, with the option of performing a DET.

SHORT PRESENTATION SESSION 2B: ASSISTED CONCEPTION

SP2B.1 How is the chance of having at least one baby following fresh ET affected by the number of mature eggs collected for each patient age cohort?

Kotrotsou Mara; Jacques Celine; Barkaoui Samya; Louzada Julio; Beaumont Lucy; Noublanche Caroline; Hickman Cristina

Apricity

Introduction: Over stimulating patients for IVF exposes patients to increased risk of OHSS; understimulating may lead to insufficient embryos, reducing the chance of a patient having a live birth (LB) following IVF. The significance of the optimal number of mature oocytes retrieved is, therefore, an important consideration for doctors and patients undergoing IVF.

Methods: Retrospective population based cohort study using the HFEA register (2000 to 2016). Fresh cycles with own eggs, own sperm were included for female age cohorts 18-34($n=140874$ cycles), 35-37($n=72209$ cycles), 38-39($n=49331$ cycles), 40-42($n=42518$ cycles), 43-44 ($n=11097$ cycles) and 45-50($n=3409$ cycles).

Results: Across all ages, the median fresh eggs collected was 9. Probability of at least one baby born after the first fresh transfer increased from 5% with 1 mature egg, to 35% with 18 mature eggs. Increasing the number of mature eggs

beyond 18 lead to a reduction in probability of LB. For patients under 35, the optimal number of eggs was 19. In all age cohorts from 35 to 50, the chance of LB continued to increase from 1 to 50 mature oocytes collected. There was a strong positive linear correlation between number of mature eggs created and number of embryos created ($y = 0.49x$, $R^2 = 0.98$). This linear correlation was not affected by patient age (NS), emphasising that age does not impact embryology KPI.

Conclusion: In this retrospective assessment of a very large data set ($n=316,029$ cycles), it is clear that, across all age groups, chance of a live birth following fresh ET is affected by the number of mature eggs collected. This impact differs by age. This conclusion should be considered by patients opting for mild stimulation, although the chance of having a baby should be taken alongside safety implications of higher stimulation strategies.

SP2B.2 Higher ongoing pregnancy rates when blastocysts with excluded cells are transferred in frozen compared to fresh cycles

Nisbett Jennifer; Wilson Paul; Akande Valentine

Bristol Centre for Reproductive Medicine

Background: The transfer of blastocysts with excluded blastomeres, is associated with lower implantation and live birth rates. The objective of this study was to explore IVF outcomes when blastocysts with excluded cells are transferred. In particular whether excluded blastomeres/fragments at the morula stage affect the clinical pregnancy rates following the transfer of fresh or cryopreserved top quality blastocysts during elective single blastocyst transfer cycles (eSBT).

Method: A retrospective review of time-lapse images (EmbryoScope+, Vitrolife) was undertaken for 199 eSBT cycles between August 2018 and April 2019. Embryo development was observed and characteristics relating to fragmentation/exclusion noted. For the purpose of this study, morulae were categorised as either Low Exclusion (LE) or High Exclusion (HE). LE was defined by $<20\%$ blastomere volume excluded i.e. ranging from no observed exclusion to a maximum of 2 blastomeres and/or some small fragments. HE exhibited $>20\%$ blastomere volume excluded i.e. 3 or more blastomeres and/or large volume of fragments; at this level the resulting trophoctoderm is visibly distorted.

Results: Where blastocysts were transferred in a fresh cycle, HE resulted in a significantly lower ongoing clinical pregnancy rate of 25% (5/20) compared with 54.1% (66/122) in the LE group ($P=0.028$) as well as a trend towards increased miscarriage rate of 37.5% (3/8) compared to 12% (9/75) in the LE group. Interestingly these findings were not observed in the frozen blastocysts where the ongoing clinical pregnancy rates were 60% (6/10) and 48.9% (23/47), and the miscarriage rates 14.3% (1/7) and 14.8% (4/27) for the HE and LE groups respectively.

Conclusions: Our findings indicate that where blastocysts exhibit HE, outcomes would be better if they are cryopreserved, and transfer deferred. It could be speculated that the vitrification process has an effect on the excluded blastomeres and the zona pellucida. However, further studies are required to validate this finding.

SP2B.3 Ovarian hyperstimulation syndrome - an underreported complication?

Malhotra Anjali¹; Rosen O'Sullivan Hannah²; Geddes-Barton Miranda¹; Glazewska Agnieszka¹; Kopeika Yuliya¹

¹Guy's and St Thomas' NHS Trust; ²St Helier Hospital

Background: Ovarian hyperstimulation syndrome (OHSS) is a complication of fertility treatment, where controlled ovarian hyperstimulation with gonadotrophin administration causes massive ovarian enlargement and acute shift of intravascular fluid into the third space. OHSS ranges from mild self limiting symptoms managed as an outpatient, to life threatening complications requiring critical care. The Royal College of Obstetricians and Gynaecologists (RCOG) suggests classifying OHSS into 4 categories - mild, moderate, severe and critical. All cases of severe and critical OHSS must be reported to the Human fertility and embryology authority (HFEA). (1-2)

Objectives: To assess if the classification of OHSS during inpatient stay was correct, whether patients were managed in the correct setting, were escalated to critical care appropriately, and whether incorrect classification prevented reporting to the HFEA.

Methods: Inclusion criteria was all patients admitted under gynaecology over a 10 year period coded as having 'ovarian hyperstimulation syndrome' in their discharge summary. Our sample size was 107 patients. We used the RCOG OHSS

classification system to correctly reclassify each patient and compared this to the classification documented by clinicians in the notes.

Results: We demonstrated that 16% of inpatients had critical OHSS, 48% severe, 18% moderate and 6% mild. Only 19% of cases were correctly classified. 61% of cases were underclassified. No patients with critical OHSS were recognised. 53 cases of severe and critical OHSS were underclassified and therefore not reported to the HFEA.

Conclusions: Given the significant number of underclassified cases of severe and critical OHSS we assume these cases were not reported to the HFEA, therefore the overall incidence of OHSS may be underestimated. No cases of severe OHSS were discussed with ITU - for one patient with critical OHSS, ITU involvement may have prevented a cardiac arrest. More education is needed for general gynaecologists on the classification of OHSS.

1. Royal College of Obstetricians and Gynaecologists. The management of ovarian hyperstimulation syndrome. Green-top Guideline No. 5. February 2016. Available from: <https://www.rcog.org.uk/en/guidelines-research-services/guidelines/gtg5/> 2. Human Fertilisation and Embryology Association. Achieving better clinical standards – best practice in OHSS. Annual conference workshop. 2018. Available from: <https://www.hfea.gov.uk/media/2558/2018-03-15-achieving-better-clinical-standards-of-care-ohss.pdf>

SP2B.4 Elective freeze all (segmentation) results in higher clinical pregnancy rates in women with high AMH

Gaudoin Marco; Ambrose Pat; Munn Linda; Roebuck Frances; Gibson Nicole; Lyon Jennifer; Fleming Richard

Glasgow Centre for Reproductive Medicine Fertility

Purpose: Numerous studies have failed to demonstrate that segmentation with mandatory blastocyst transfer improves outcomes compared with fresh embryo transfer, although this latter group has an increased risk of ovarian hyperstimulation syndrome (OHSS). Our internal data showed that GnRH-antagonist controlled cycles with HCG triggering for women with high AMH (≥ 30 pmol/L) had poorer fresh clinical pregnancy rates compared with older women with lower AMH despite the former group theoretically being a better prognosis group. Hence, at the start of 2019, we changed clinical practice to segmented cycles in these women.

Methods: All women with high AMH used GnRH-antagonist control and underwent blastocyst transfer in a single centre. Historical data from the patients' first fresh embryo transfer in 2017 and 2018 (N = 78, Group-Hist) was compared with prospective data from January-August 2019 who underwent segmentation and their subsequent first natural cycle-frozen embryo transfer (N = 23, 21 embryo transfers, Group-Segn).

Results: There was no difference in age, BMI, number of eggs, 2PN embryos or embryos transferred between the two groups. Two women (9%) in Group-Segn had no blastocysts suitable for freezing. These two women are included in the subsequent analysis. Group-Segn had significantly higher positive pregnancy rates (65% vs. 32%, $P=0.007$), clinical pregnancy rates (56% vs. 23%, $P=0.004$) and implantation rates (54% vs. 26%, $P=0.01$).

Conclusions: Despite the dataset being quite small, segmentation in high responder patients with GnRH-antagonist control resulted in significantly higher pregnancy and implantation rates. The low implantation rate in fresh treatment cycles may be related to higher progesterone levels in the follicular phase in GnRH-antagonist cycles (Bosch et al, 2010). Segmentation and subsequent frozen embryo transfer allows the use of Buserelin-only triggering, thus minimising the risk of OHSS, but patients must be counselled that there may be no embryos suitable for vitrification.

SP2B.5 Endometrial assessment and personalised plan in the treatment of unexplained repeated embryo implantation failure: one year experience

Rahmati Mona; Ripanelli Alessandra; Smilgeviciute Vaida; Nair Shailaja; Gill Ajit; Balet Richard; Ahuja Kamal; Macklon Nick

London Women's Clinic

Background: The current management of recurrent implantation failure is largely based on empirical treatments aim at improving endometrial receptivity. However, the emergence of tests of different aspects of endometrial function provides the opportunity to direct therapies to address underlying pathologies. We present the first year clinical outcomes achieved from a unit dedicated to the investigation and treatment of patients with unexplained repeated embryo implantation failure.

Methods: Patients referred had undergone at least three embryo transfer procedures without success. Timed endometrial biopsy was performed after 5 days of luteal support in a hormone substituted cycle. Biopsies were subject to endometrial dating by gene expression (ERA test, iGenomix, Valencia) to endometrial immune profiling including the recruitment and activation of the uterine Natural Killer cells (Matrice Lab Innove, Paris).

Based on the results, a management plan focusing on optimizing endometrial preparation and embryo transfer was proposed to the referring clinician.

Results: 133 patients with an average of 3.9 blastocysts previously transferred underwent investigation. Results from 73 are currently known and those from 40 will become available by the end of 2019. 91% of the patients tested revealed at least one abnormal test outcome. No correlation was observed between the results of the two test profiles. The outcome of the first attempt with personalised plan is available for 44 patients, showing an implantation rate of 55% and an ongoing pregnancy rate of 37% per transferred embryo. Those who did not achieve a subsequent implantation showed a higher prevalence of normal endometrial profiling compared to those who conceived on the implemented treatment plan.

Conclusions: This uncontrolled pilot study suggests that treatment based on endometrial diagnostics might improve outcomes compared with empirical management. Although controlled studies are required, this approach provides the opportunity to test targeted rather than blind interventions in randomized controlled trials.

SP2B.6 Protective effects of omega-3 fatty acids on cyclophosphamide induced fertility alterations

Nnamonu Emmanuel

Federal College of Education, Eha-Amufu, Enugu State Nigeria

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 foetal numbers, litter size, foetal weight, copora lutea number, foetal 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 foetal weight, copora lutea number, foetal 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.

SHORT PAPER SESSION 2C: FEMALE REPRODUCTIVE TRACT

SP2C.1 Dynamic properties of endometrial macrophages regulate tissue homeostasis in the uterus

Mann Elizabeth

University of Manchester

Background: Dysregulated tissue degradation, repair and remodelling in the uterus can lead to structural abnormalities and fibrosis, causing reproductive dysfunction. In other tissues, macrophages are central to tissue remodelling and repair. However, the ontogeny and functions of macrophages within the uterus are poorly defined, despite dysregulated macrophage recruitment and function driving fibrosis in other tissues.

Methods: Ontogeny of murine endometrial macrophages was defined 8-20 weeks after reconstitution of irradiated mice (uterus lead-shielded) with congenic bone marrow to enable tracking. Macrophages were characterised by multi-

parameter flow cytometry at different stages of the estrous cycle.

Results: Mature macrophages were predominant within uterine immune cells, and were present at all stages of the estrous cycle, comprising two distinct populations based on expression of MHC class II. MHC class II- macrophages expressed features indicating involvement in tissue remodelling and repair, including expression of high levels of the IL-4 receptor, CSF-1 receptor, CD206 and resistin-like molecule α . Endometrial macrophages did not proliferate in situ but a substantial proportion were derived from circulating monocytes in a manner dependent on the CCR2 chemokine receptor. Numbers of monocytes were profoundly increased during metestrus (oestrogen/progesterone low); these monocytes were highly proliferative, indicating the potential for local expansion and maintenance. Ccr2^{-/-} mice that are deficient in blood monocytes contained tissue-derived macrophages that were self-renewing as indicated by their high levels of proliferation, and expressed higher levels of the IL-4R compared to wild-type mice.

Conclusions: The estrous cycle is marked by dynamic changes in populations of macrophages/monocytes that are associated with varying dependence on replenishment from the bloodstream versus local renewal. Recruitment of monocytes is CCR2-dependent with expansion of monocytes during metestrus controlled by local proliferation. These events, including generation of macrophages with distinct roles in tissue repair and remodelling, are likely crucial for receptivity to implantation and prevention of fibrosis.

SP2C.2 Can we detect endometrial markers of hypoxia in women using Magnetic Resonance Imaging (MRI) to improve management of heavy menstrual bleeding (HMB)?

Reavey Jane¹; Walker Catherine¹; Murray Alison¹; Nicol Moira¹; Critchley Hilary¹; Kershaw Lucy²; Maybin Jacqueline¹

¹MRC Centre for Reproductive Health, Queen's Medical Research Institute; ²Edinburgh Imaging Facility

Background: HMB is a common, debilitating condition requiring improved therapeutics. Better understanding of menstrual physiology and pathology is essential. There is in vitro evidence that endometrial hypoxia is present at menstruation and drives endometrial repair. Detection of hypoxia in vivo remains elusive. We hypothesized that:

1. Markers of endometrial hypoxia can be detected in vivo and in vitro in women with normal menstrual bleeding (NMB) at menstruation.
2. Women with HMB have decreased markers of endometrial hypoxia at menstruation compared to those with NMB.

Methods: With ethical approval and consent, participants (n=24) underwent measurement of menstrual blood loss (alkaline haematin method: NMB <80ml) and attended for two MRI scans; during menstruation and the early/mid-secretory phase. The protocol included dynamic contrast-enhanced MRI (DCE-MRI) and T2* quantification. Deoxyhaemoglobin increases local magnetic field inhomogeneity which decreases T2*. An endometrial sample was also collected to assess in vitro hypoxic markers by qRT-PCR: hypoxia-regulated repair factors (ADM, VEGFA, CXCR4) and lactate dehydrogenase (LDHA).

Results: In vivo imaging revealed women with NMB had reduced T2* (p=0.005) at menstruation consistent with hypoxia at menses. Plasma flow (F_p) was increased at menstruation compared to the non-menstrual phase (p=0.0005). In vitro findings in women with NMB showed reduced ADM, VEGF-A and LDHA in menstrual compared to non-menstrual endometrium (p=0.008; p=0.004; p=0.012). Comparing women with NMB and HMB, in vivo imaging showed no difference in T2* at menstruation. Endometrial F_p and plasma volume (v_p) were reduced at menstruation in women with HMB compared to NMB (p=0.004; p=0.041). This may reflect delayed initiation of endometrial repair.

Conclusions: These in vivo data support the presence of hypoxia in the endometrium of women with NMB. Women with HMB may have a defective hypoxic response at menstruation. Non-invasive detection of these hypoxic markers may aid diagnosis and provide novel therapeutic targets for HMB.

SP2C.3 The role of GW4869 in extracellular vesicles (EVs) biogenesis and its effect on embryo-endometrial implantation

Binti Liaqat Ali Khan Norhayati¹; Basatvat Shaghayegh¹; Elliott Sarah¹; Heath Paul²; Fazeli Alireza¹

¹Academic Unit of Reproductive and Developmental Medicine Department of Oncology & Metabolism, University of Sheffield, UK; ²Sheffield Institute for Translational Neuroscience, University of Sheffield

Background: Embryo implantation involves intercellular communication triggered by signals exchanged between the embryo and the endometrium (1). This communication may be mediated by extracellular vesicles (EVs) which contain lipids, proteins, DNAs and RNAs (2). Inhibition of EVs biogenesis by GW4869; a neutral sphingomyelinase inhibitor may interfere with or even cause a breakdown of the cell-cell communication, thus affect embryo implantation (3). Aim: We aimed to investigate the role of GW4869 on EVs' biogenesis in both endometrial and trophoblast cells and its effect on embryo endometrial communications. Hypothesis: Inhibition of EVs biogenesis reduces the JAr spheroids -- RL95-2 binding affinity.

Methods: JAr cell spheroids representing the embryo and RL95-2 cell representing the receptive endometrium were both introduced twice to 5µM, 10µM, and 20µM GW4869 in 0.1% DMSO and incubated for 1 hour respectively in each session. The conditioned media was collected, and EVs were isolated using size exclusion chromatography (SEC) and further used for nanoparticle tracking analysis (NTA). The possible toxicity of GW4869 and DMSO on the cells were examined using a cell viability/toxicity assay. For the binding assay, 35 JAr spheroids were incubated for 1 hour with a monolayer of RL95-2 containing GW4869. The binding percentages were calculated and statistical analyses carried out.

Result: There was a reduction in the particle counts (EVs) in GW4869 treated group compared to the control groups, but no significant reduction in cell viability. The percentage JAr spheroids bound to endometrium in complete Dulbecco's Modified Eagle Media (DMEM), EV-depleted DMEM, Fetal Bovine Serum (FBS)-free DMEM and FBS-free DMEM media+GW4869 was 98.78%±0.91, 93.33%±2.28, 90.89%±2.49 and 61.67%±3.75 (Mean±SEM); respectively.

Conclusion: GW4869 inhibits the biogenesis of EVs and significantly (ANOVA, p<0.001) reduce the JAR spheroids endometrial binding affinity. It seems EVs are involved in intercellular communication between mother and the baby at the early stages of the implantation

1) Zhang S, Lin H, Kong S, Wang S, Wang H, Wang H et al. Physiological and molecular determinants of embryo implantation. Mol Aspects Med, 2013. 34(5): p. 939-980. 2) Kalra H, Drummen GP, Mathivanan S. Focus on Extracellular Vesicles: Introducing the Next Small Big Thing. Int J Mol Sci, 2016. 17(2):170. 3) Menck K, Sönmezer C, Worst TS, Schulz M, Dihazi GH, Streit F et al. Neutral sphingomyelinases control extracellular vesicles budding from the plasma membrane. J Extracell Vesicles, 2017. 6(1):1378056 NEEDS TO BE ADDED ON PROGRAMME FOR SPEAKER: (Academic unit of Reproductive and Developmental Medicine, Department of Oncology and Metabolism, Level 4, Jessop Wing, University of Sheffield1, Faculty of Dentistry, Universiti Teknologi MARA (UiTM), Malaysia2 Same goes to my supervisor. (Academic unit of Reproductive and Developmental Medicine, Department of Oncology and Metabolism, Level 4, Jessop Wing, University of Sheffield1Institute of Biomedicine and Translational Medicine Department of Pathophysiology, University of Tartu, Tartu, Estonia4

SP2C.4 The effect of prokineticin 1 on the angiogenesis in the endometrium during pregnancy

Goryszewska Ewelina; Baryla Monika; Kaczynski Piotr; Wacławik Agnieszka

Institute of Animal Reproduction and Food Research of Polish Academy of Sciences

Prokineticin 1 (PROK1), is also called as an endocrine gland-derived vascular endothelial growth factor (EG-VEGF). Studies in humans have shown that PROK1 plays a key role in regulating pregnancy-related processes such as angiogenesis, proliferation and regulation of the immune response. In the female endometrium, expression of PROK1 and its receptor (PROKR1) is increased during implantation and placenta development in the first trimester of pregnancy. The aim of the present study was to determine PROK1 effect on proliferation and capillary-like network formation of porcine endometrial endothelial cells.

Uteri were collected from gilts on day 15 (n=10) of the pregnancy. Endometrial tissue was digested by dispase and next by the collagenase. The cell mixture was treated by Dynabeads® CD31 Endothelial Cell according to manufacturers' protocol. To determine the PROK1 effect on proliferation, endothelial cells were seeded into a 96-well plate and cultured to around 40% confluence. Then, the cells were incubated 24 hours with 40 nM PROK1 in the presence or absence of 1 µM PROK1 receptor antagonist (PC7). Cell proliferation was assessed by colorimetric method. To determine the PROK1 effect on the capillary-like network formation, endothelial cells were seeded on µ-Slide dishes and incubated 6 h with 40 nM PROK1 together/without 1 µM PC7. Two-way ANOVA followed by Tukey post-tests was used to analyze obtained results.

Proliferation of endometrial endothelial cells was increased after incubation with PROK1 on the 15 day of pregnancy ($p < 0.05$). This effect was abolished after using PC7. Moreover, PROK1 stimulated capillary-like network formation. A statistically significant increase was observed in 15 of the 20 parameters describing angiogenesis ($p < 0.05$). Co-treatment with PC7 diminished the stimulating effect of PROK1.

Results of the present study indicate that PROK1 are important factors involved in the angiogenesis in the porcine endometrium during pregnancy.

SP2C.5 Tight junction decoupling from actomyosin in receptive endometrial epithelium is regulated by protein O-GlcNAcylation

Shelmerdine Eva¹; Ruane Peter¹; Brosens Jan²; Kimber Sue¹; Brison Daniel³; Westwood Melissa¹; Aplin John¹

¹University of Manchester; ²Warwick Medical School, University of Warwick; ³Old St Mary's Hospital

Aims & Objectives: Understanding the mechanisms of altered cell function that lead to endometrial receptivity is vital for improving ART. In primary endometrial organoids, epithelial tight junction organisation in response to hormone-induced receptivity was examined. Using an endometrial cell line we investigated junctional organisation in response to modulation of glucose and downstream protein O-GlcNAcylation.

Methods: Endometrial organoids were treated with estrogen, progesterone and cyclic adenosine monophosphate (cAMP). The endometrial epithelial Ishikawa cell line was treated with different glucose levels, an inhibitor of protein O-GlcNAcylation (OSMI-1) and an inhibitor of Rho kinase (ROCK) signalling (Y27632). Samples were analysed by fluorescence microscopy using antibody markers and cell structure-specific dyes.

Results: In endometrial organoids, active ser19-phosphorylated myosin light chain (pMLC) was found to co-localise with zona occludins (ZO-1) at apical intercellular tight junctions. Treatment of organoids with hormones to mimic the receptive phase abolished pMLC junctional localisation. In Ishikawa cells grown to confluence in 5mM glucose, pMLC also localised to tight junctions, however in 17mM glucose this localisation was lost. pMLC localisation was not glucose-responsive in organoids. In Ishikawa cells, OSMI-1 treatment rescued pMLC co-localisation with ZO-1 in 17mM glucose, while Y27632 treatment led to loss of pMLC and ZO-1 from junctions.

Conclusions: Endometrial epithelial tight junction coupling to contractile actomyosin through MLC may be crucial for receptivity, and diabetes could affect receptivity through protein O-GlcNAcylation downstream of dysregulated glucose levels. Decreased pMLC likely loosens tight junctions, allowing apical localisation of cell-cell adhesion proteins required for embryo attachment while also permitting epithelial breaching by invasive trophoblast during implantation. MYPT1 is a highly O-GlcNAcylated protein that is regulated by ROCK and targets MLC for dephosphorylation, and thus may serve as a glucose-sensitive molecular switch for endometrial epithelial receptivity.

SP2C.6 Cystic ovary disease (COD) impairs functional morphology of the bovine oviduct

Scully Deirdre¹; Dockery Peter²; Reese Sven³; Kolle Sabine¹

¹University College Dublin; ²National University of Ireland, Galway; ³Ludwig Maximilians University

Background and objectives: Cystic ovary disease (COD) is a common cause of subfertility in humans and animals. To date, the effects of COD on the microarchitecture of oviducts are largely unknown. Therefore, the aim of this study was to analyse the effects of COD on the functional morphology of the oviduct as well as on gameto-maternal interactions.

Methods: For this purpose, oviducts affected by follicular cysts (FC n=22), follicular cysts with luteinisation (FCL n=6) and luteal cysts (LC n=26), as well as controls (n=44), were investigated by stereomicroscopy, histochemistry, scanning (SEM) and transmission electron microscopy (TEM). A unique live cell imaging (LCI) system was used to capture real time videos of gameto-maternal interactions under near in vivo conditions.

Results: The incidence of COD in slaughtered cows was 3.1%. COD was more likely to affect the right ovary ($p = 0.02$, Binomial test) and was correlated with endometritis ($p < 0.0001$, Chi-square test). The ampullar folds from cows with COD were significantly thicker than folds in the control ampulla ($p = 0.02$, ANOVA with Dunnett's). Oviducts from cows with FC and FCL were characterized by increased proportions of non-protruding secretory cells (SC) as compared to the control ampulla ($p < 0.001$, ANOVA with Dunnett's). As shown by TEM tubal cells of cows affected by LC revealed highly

active rough endoplasmic reticulum and accumulation of secretory granules. Synthesis of glycoproteins and acidic mucopolysaccharides in the tubal epithelium was significantly increased in FC and FCL as compared to controls ($p < 0.05$, ANOVA with Dunnett's). Sperm binding and motility, as well as cumulus-oocyte-complex binding, were maintained in ampullae from cows with COD.

Conclusion: Our results demonstrate that tubal morphology and function are impaired by COD. This includes an altered ratio of secretory cells and increased secretion of glycoproteins leading to impaired tubal metabolism and nutrition of the gametes and the embryo.

SHORT PAPER SESSION 2D: EMBRYO DEVELOPMENT

SP2D.1 Cytokinesis in early mouse embryo

Vasilev Filip¹; FitzHarris Greg²

¹Centre de Recherche du Centre Hospitalier de l'Université de Montréal (CRCHUM); ²University of Montreal, Centre de Recherche du Centre Hospitalier de l'Université de Montréal (CRCHUM)

Introduction: Early mammalian embryos frequently possess binucleated cells, suggesting a failure of cytokinesis, the final step of cell division causing the physical separation of the two new daughter cells. In somatic cells cytokinesis comprises the formation of a contractile ring that drives the ingression of the cleavage furrow to bisect the cell at the equator. At the end of cytokinesis the contractile ring transforms into a so-called midbody structure also comprising stabilized microtubules to maintain contact between the newly nascent cells.

Results and methods: By use of live cell imaging here we observed that the contact point of the two dividing blastomeres defines the rate of contraction of the cytokinetic furrow. As a result, the side that is free of any contact with the neighboring blastomere contracts more dramatically than the contralateral cell-contacting side. Using micromanipulation, we show that this asymmetric mode of cytokinesis is critically dependent upon the neighboring blastomere. Secondly, with time-lapse confocal microscopy we demonstrate the recruitment of anillin in the plasma membrane during the cytokinetic furrow ingression, which ultimately forms a focused midbody structure on the intercellular microtubule bridge. When one of daughter cells undergoes a subsequent division, the microtubule bridge disassembles, and concomitantly the anillin-positive structure is apparently expelled from the cell and forms an extracellular structure positive for plasma membrane components and the microtubule crosslinker PRC1. By tracking this extracellular structure in 4D live imaging, we have evidence that it can persist for at least two divisions before it disappears.

Conclusions: Cytokinesis in the mammalian embryo is influenced by the cell-cell contact during the cleavage divisions. After each blastomere division, the midbody remnant is released before the onset of the next division. Future experiments will address the question whether the midbody and/or the midbody remnant carry signals that might help pattern the early embryo.

SP2D.2 The metabolic and developmental impact of microfluidic culture on preimplantation bovine embryo development *In vitro*

McKeegan Paul¹; Pensabene Virginia²; Actis Paolo²; Savva Constandinos²; Picton Helen²

¹Hull York Medical School; ²University of Leeds

Microfluidic devices recreate the microphysiological environment of the mammalian oviduct by controlling fluidic shear stress in small media volumes, potentially improving *in vitro* embryo development. We recently reported a 400nl chamber polydimethylsiloxane device for murine embryo culture¹. Here we present data on the microfluidic culture of bovine embryos.

Abattoir-derived bovine IVP embryos were produced according to established protocols². Initial experiments cultured embryos in a novel 2800nl Large Chamber device (LC). To define device limitations, cleaved bovine embryos were cultured to the blastocyst stage in groups of 5, 10 or 20 (LC5, LC10, LC20) or in control groups of 20 embryos in 20µl

SOFaBSA microdrops under oil (CT20) at 39°C, 5%CO₂/5%O₂/90%N₂. Day 7 blastocysts were removed to individual 4µl drops for 24h to profile glucose, pyruvate, lactate² and amino acid turnover^{3,4}.

Microfluidic culture supported similar blastocyst formation rates to controls (LC5: 22±3.5%, n=30, LC10: 25±4.8%, n=22, LC20: 18±3.2%, n=21, control: 23±3.1%, n=25, p=0.48). Blastocyst glucose, pyruvate, lactate and amino acid turnover were similar between devices and controls, regardless of group size (p>0.05). This suggests the device supports embryo development in small groups without adverse effects. Groups of 5 embryos were therefore adopted in subsequent experiments.

To evaluate the potential beneficial effect of reduced culture volume, an 800nl Small Chamber (SC) device was developed. Cleaved bovine embryos were cultured to day 7 in SC and LC devices versus control groups of 5 embryos in 5µl drops (CT5). Blastocyst development rate was highest in SC groups (CT5: 11±2.3%, n=19, SC: 27±5%, n=15, LC5: 22±3.5%, n=30, p=0.04). SC and CT5 blastocysts had significantly higher glucose consumption than LC blastocysts (CT5: 39.01±5.55pmol/embryo/hr, n=5, SC: 39.38±6.10pmol/embryo/hr, n=16, LC5: 15.52±3.28pmol/embryo/hr, n=7, p<0.001).

SC devices provide a viable culture system for small numbers of mammalian embryos. Further studies will explore the impact of device culture on human embryos.

1 Colucci F., McKeegan P., Picton H., Pensabene V. (2018). Mouse embryo assay to evaluate polydimethylsiloxane (PDMS) embryo-toxicity. Conf. Proc. IEEE Eng Med Biol Soc.

2 Guerif, F., McKeegan, P., Leese, H. J., & Sturmey, R. G. (2013). A simple approach for CONsumption and RElease (CORE) analysis of metabolic activity in single mammalian embryos. PLoS One, 8(8), e67834. doi:10.1371/journal.pone.0067834

3 Hemmings, K.E., Leese, H.J., Picton, H.M (2012). Amino Acid Turnover by Bovine Oocytes Provides an Index of Oocyte Developmental Competence In Vitro. BIOLOGY OF REPRODUCTION. 86 (5):165, 1-12. DOI 10.1095/biolreprod.111.092585

4 Sturmey, R.G., Bermejo-Alvarez, P., Gutierrez-Adan, A., Rizos, D., Leese, H.J. and Loneragan, P. (2010). Amino Acid Metabolism of Bovine Blastocysts: A Biomarker of Sex and Viability. Molecular Reproduction & Development 77:285-296

SP2D.3 Effects of different infra-red laser-assisted hatching (LAH) protocols on pre-implantation mouse embryos and embryo attachment

Liu Yaqiong; Jones Celine; Coward Kevin

University of Oxford

Background: Although assisted hatching has been used in clinics for more than 25 years, and new methodologies are continually being developed, there is a distinct lack of literature relating to the basic safety and specificity of laser-assisted protocols [1, 2].

Methods: In total, 1481 E2.5 mouse embryos were treated by 3 different LAH methods (Complete LAH, Partial LAH, and ZP thinning) and cultured for 2 days in vitro. Embryo development, hatching pattern[3, 4], embryo cell composition (inner cell mass [ICM] and trophectoderm [TE]), cellular apoptosis, and the expression levels of key genes were detected and compared between different groups at E4.5. Furthermore, a two-dimension implantation model, featuring Ishikawa cells, was used to test embryo attachment capability.

Results: No significant differences were detected in embryo development, cell number count, cell apoptosis index or the expression of caspase-3 and heat shock protein-70. However, complete LAH improved the initiation of embryo hatching in both fresh and frozen-thawed embryos (81.1% [210/259] and 91.9% [119/240], respectively). Furthermore, the rate of '8'-shape hatching was significantly higher with complete LAH (75% [105/140]) than in any other group (P<0.05). At the gene expression level, an apoptosis-related gene (Bax) was down-regulated (by 37%) following complete LAH treatment. In the implantation model, complete LAH impeded the completion of hatching; however, zona thinning slightly improved the rate of fully hatched embryos and the stability of embryo attachment during the co-culture period.

Conclusion: Complete LAH could improve the initiation of embryo hatching and change embryo hatching behaviors, without impairing embryo development and cell differentiation, or inducing cell apoptosis and heat shock stress. However, this technique may change the gene expression of Bax, an apoptosis-related gene, and impede the completion of hatching. Furthermore, zona thinning might be a more efficient method with which to improve full hatching and attachment.

1. Davidson, L.M., et al., Laser technology in the assisted reproductive technology laboratory: a narrative review. Reproductive biomedicine online, 2018.
2. Hammadeh, M.E., C. Fischer-Hammadeh, and K.R. Ali, Assisted hatching in assisted reproduction: a state of the art. Journal of assisted reproduction and genetics, 2011. 28(2): p. 119-128.
3. Onodera, Y., et al., The location of "8"-shaped hatching influences inner cell mass formation in mouse blastocysts. PloS one, 2017. 12(4): p. e0175150.
4. Yan, Z., et al., Eight-shaped hatching increases the risk of inner cell mass splitting in extended mouse embryo culture. PloS one, 2015. 10(12): p. e0145172.

SP2D.4 Sex sorting of bovine sperm leads to compromised development of the early embryo

Steele Heather¹; Makri Dimitra²; Maalouf Walid²; Reese Sven³; Koelle Sabine¹

¹University College Dublin, Ireland; ²University of Nottingham, UK; ³University of Munich, Germany

Background: Sex-sorting of bovine sperm has been introduced in assisted reproduction to increase the number of female calves in the dairy industry. However, it results in significantly reduced conception rates when used for artificial insemination (AI) [1,2]. The cause is currently unknown. Therefore, the aim of this study was to investigate how the use of sexed sperm affects early embryo development.

Methods: Bovine oocytes were aspirated from ovaries from cows immediately after slaughter. Oocytes were matured in vitro, fertilized with conventional and sexed semen of the same bulls (n=5) and cultured for 7 days until the blastocyst stage (n=360). EmbryoScope time-lapse videomicroscopy was applied for quantitative analyses of embryo development. Dual immunofluorescence (DAPI/TMR red) for detection of apoptosis in day 7 embryos was also performed.

Results: Embryos derived from sexed-semen were significantly more likely to fail to cleave (n=58, binomial test, p<0.05), to arrest at the 4-cell stage (n=6, binomial test, p<0.05) and to fail to develop to the blastocyst stage as compared to controls (n=188, binomial test, p<0.001). Further to that, the survival time of embryos derived from sexed-semen was significantly shorter as compared to controls (HR 1.54, Cox proportional hazards regression p<0.001). The relative risk for sexed-sperm derived embryos to shrink or fuse cells during development was 1.71 times higher as compared to controls. The percentage of apoptotic cells was similar in embryos derived from sexed or conventional sperm. However, the percentage of apoptotic cells in compact blastocysts was significantly higher as compared to expanding blastocysts (n=107, generalized estimating equation, p<0.001) and hatching blastocysts (n=19, generalized estimating equation, p<0.001).

Conclusions: Sexing of sperm results in compromised early embryonic development. Thus, the process of sex-sorting affects sperm in a manner which persists beyond fertilization. This highlights the fact that sperm integrity plays a major role in embryo quality.

1. Hutchinson IA, Shalloo L, Butler ST. Expanding the dairy herd in pasture-based systems: The role of sexed semen use in virgin heifers and lactating cows. J Dairy Sci 2013; 96:6742-6752.
2. Carvalho JO, Sartori R, Rodello L, Mourao GB, Bicudo SD, Dode MAN. Flow cytometry sex sorting affects bull sperm longevity and comprises their capacity to bind to oviductal cells Livestock Science 2018;207:30-37.

SP2D.5 Embryo Ranking Intelligence Classification Algorithm (ERICA): a deep learning-based clinical assistant for blastocysts selection

Chavez-Badiola Alejandro¹; Flores Saiffe Farías Adolfo¹; Mendizabal-Ruiz Gerardo²; Drakeley Andrew³

¹New Hope Fertility Center; ²Universidad de Guadalajara; ³Hewitt Centre for Reproductive Medicine

Objective: Develop and assess ERICA (Embryo Ranking Intelligence Classification Algorithm), an AI that ranks blastocysts according to prognosis.

Design: Retrospective morphometric study to evaluate ERICA.

Materials and methods: We used a database of 1265 blastocyst micrographs obtained from three different clinics and five different laboratory settings (two laboratories used two different microscopes presets). All images were taken during day five or six after fertilization and before any intervention was made on embryos (i.e., biopsy, cryopreservation, or transfer). ERICA, after image pre-processing, extracts 94 features from each image. The feature

extractor was designed to quantify known predictors of embryo viability (e.g., size, shape, and observable inner mass), and other features not identifiable under standard microscopy. ERICA was trained with these features (80% of the dataset) and tested (10% of the dataset) to predict blastocysts' prognosis (PGT-A result of euploidy or b-hCG $\geq$ 20). We used 10% of the database as the testing set (83 embryos corresponding to 19 cycles) with known PGT-A results, to test the PGT-A prediction ability on each embryo and its ranking on each cycle.

Results: ERICA obtained a positive predictive value of 0.79 and an AUC of 0.74 for predicting euploidy on the testing set. The ranking of ERICA was better at predicting euploidy than the results of random prediction as well as predictions made by two senior embryologists based on the images from 19 cycles (83 embryo images) ($p < 0.05$). ERICA was also able to find an euploid embryo in 78.9% of the tested cycles (15 out of 19) and 94.7% of finding at least one euploid within the first two, also overcoming aleatory and the two embryologists.

Conclusions: The evidence presented in this work justifies and supports prospective study where ERICA could be tested with new data and with different laboratory settings and microscopes.

SP2D.6 Non-invasive chromosome screening reveals high concordance rate when using MALBAC technique for whole genome amplification for preimplantation genetic testing for aneuploidies

Odia Rabi¹; Ben Nagi Jara¹; Vinals-Gonzalez Xavier¹; Wang Jason²; Hensinger Brandon²; Serhal Paul¹

¹Centre for Reproductive and Genetic Health; ²Yikon Genomics

Background: Several developments in non-invasive preimplantation genetic testing for aneuploidies (PGT-A) have been achieved with the use spent culture media (SCM) with varying concordances. Non invasive PGT-A can prevent false positive results compared to trophectoderm (TE) biopsy which is associated with mosaicism. Nonetheless, key aspects such as the degree of concordance that is acceptable for clinical application is still being investigated. Aims: This pilot study aimed to unveil the effectiveness of non-invasive screening of SCM using multiple annealing and looping based amplification cycles (MALBAC) technique for whole genome amplification and compare the ploidy results to the current gold standard technique, trophectoderm (TE) biopsy.

Methods: Vitriified donated blastocysts with known chromosomal status following TE biopsy were thawed and re-cultured overnight in independent 15 μ L drops of culture medium. Embryos were then allowed to perish and the SCM was collected for processing. MALBAC technique and next generation technology were used for amplification and aneuploidy detection using ChromInstTM on Illumina platform (Yikon Genomics), respectively.

Results: A total of 30 blastocysts vitriified after PGT-A were used: one euploid, 27 aneuploid and two abnormal mosaic. The concordance rate for embryo ploidy category was 26/30 (86.6%, 95% CI 70.3-94.7) and 19/26 (73%, 95% CI 53.9-86.3) when taking into account the actual chromosomes affected. Two mosaic and two aneuploid embryos were classified as "euploid", resulting in a false positive rate of 13.3 (95%, 5.3-29.7).

Conclusions: The results from this pilot study using MALBAC technology show that non-invasive testing of SCM is robust for ploidy detection at the blastocyst stage. With concordance rate of almost 90%, this may be a not-so-far away technology which could revolutionize ART laboratories. Whole embryo biopsy can be undertaken in the future to determine accurately the ploidy status of embryos with dis-concordant results.

SHORT PAPER SESSION 2E: OVARY & FOLLICULOGENESIS

SP2E.1 Ovarian INSL3 acts as a feedforward drive promoting the growth and steroidogenesis of healthy antral follicles

Anand-Ivell Ravinder; Dai Yanzhenzi; Ivell Richard

University of Nottingham

Background: In the female mammal, the peptide hormone INSL3 is uniquely made by the theca interna cells of healthy growing antral follicles. We have previously shown that INSL3 acting in an autocrine/paracrine manner on its specific GPCR receptors, RXFP2, also on theca cells, is essential for the production of the key steroid precursor androstenedione [1]. Using primary cultures of mid-follicular phase theca cells from bovine follicles, we now show that the INSL3-RXFP2 system is a major component, extending the classical 2-cell hypothesis, to explain the growth and function of antral follicles during the follicular phase [2].

Methods: Primary cultures of mid-follicular phase (6-8mm diameter) bovine ovarian theca cells, which will not have seen the LH surge in vivo, were obtained from slaughterhouse ovaries and treated or not with various steroid agonists and antagonists, as well as gonadotropins. We have shown that this model system closely reflects the physiology of the equivalent human cells. As end-points, theca cell production of INSL3 was measured using specific time-resolved immunoassay (TRFIA); alternatively, the activity of transiently transfected INSL3 promoter-reporter constructs was assessed.

Results and Discussion: The key results indicate that both androstenedione (from theca cells) and estradiol (from granulosa cells), both acting through estrogen receptors, exert a feedforward pressure on INSL3 production; androgens have little or no effect. Importantly, whereas low level LH, as pertains in the early follicular phase, is stimulatory, the high concentration found during the LH surge is inhibitory. Thus the INSL3-RXFP2 system drives a feedforward loop as the follicle grows, with increasing androstenedione and estradiol potentiating the kinetics, until the LH surge, when the system is abruptly and effectively switched off, accompanying luteinisation. This study also underscores the importance of secreted INSL3 in the female as a reliable biomarker of antral follicle growth and health.

References: [1] Glister C, Satchell L, Bathgate RA, Wade JD, Dai Y, Ivell R, Anand-Ivell R, Rodgers RJ, Knight PG. (2013) A functional link between Bone Morphogenetic Protein and Insulin-like Peptide 3 signaling in modulating ovarian androgen production. *Proc Natl Acad Sci USA* 110: E1426-E1435. [2] Dai Y, Ivell R, Anand-Ivell R (2017) Theca cell INSL3 and steroids together orchestrate the growing bovine antral follicle. *Front Physiol* 8:1033.

SP2E.2 The role of vascular androgen receptors in both follicle development and ovarian hyperstimulation syndrome

Lin Tiffany; Chen Weixi; MacDonald Virginia; Spears Norah; Murray Joanne

University of Edinburgh

Introduction: The function of ovarian vascular androgen receptors (vAR) is unknown. We hypothesized that vAR modulate vascular permeability thereby influencing the supply of nutrients to follicles.

Aims: To determine: (1). the role of vAR on folliculogenesis; (2). if vAR modulate the amount of global oedema resulting from OHSS-induction; and (3). if endothelin-1 receptor (ET_A and ET_B) expression is associated with changes in vAR expression and OHSS-induction.

Methods: Wildtype (WT) mice and mice with AR genetically ablated from the endothelial- (VEARKO), vascular smooth muscle-cells (SMARKO) or both cell types (DOUBLE) were used. Control animals were injected with 5 IU PMSG on Day 2 whilst OHSS was induced by injecting 20 IU PMSG on Days 1, 2 and 3. All animals were injected with 7.5 IU hCG on Day 4 and killed 8 hrs later. All animals were weighed daily. Ovaries were either fixed for histology or processed for qPCR.

Results: (1). Compared to WT (n=6) and VEARKO (n=6) control ovaries, the total numbers of follicles SMARKO (n=6) and DOUBLE (n=6) control ovaries were reduced (P<0.05): this was accounted for by a reduction in primordial follicles. The proportions of preantral and antral follicles identified as being unhealthy were approximately double in all 3 ARKO genotypes compared to the WT (P<0.05). (2). Body weight change, a surrogate measure of global oedema, was less in VEARKO mice (3.1±3.2%; n=7) compared to WT (5.8±2.5%; n=9), SMARKO (7.0±3.4%; n=11) and DOUBLE (4.8±1.6%; n=11) mice; that is, the vasculature of VEARKO mice was less leaky. (3). ET_B expression was increased following OHSS-induction in VEARKO (P=0.004; n=3) and DOUBLE (P=0.014; n=3) ovaries. ET_B activation is associated with decreases in vascular leakage[1].

Conclusion: vAR have a role in maintaining the health of growing follicles. The vAR of the endothelial cells appear to alter ET_B expression to modulate vascular permeability.

SP2E.3 Does basal antral follicle count correlate with number of oocytes collected?

Schwab Stephanie; McGeeney Shelley; Robinson Michele; Morris Guy; Mavrelos Dimitrios

Reproductive Medicine Unit, University College London Hospital

Introduction: The current theory of folliculogenesis(1) suggests that all follicles available for recruitment are visible on ultrasound at the point when ovarian stimulation is applied. This implies a tight correlation between the antral follicle count (AFC) on D3 of a stimulation cycle and the eventual number of follicles collected. Our aim was to test this hypothesis.

Methods: Prospective cohort study of women undergoing ovarian stimulation between January and April 2019 in a central London teaching hospital. We included women who were prescribed an antagonist protocol with 450iu gonadotrophin daily injection and a 10,000unit bhCG trigger. We excluded women undergoing fertility preservation cycles as their response is known to be different (2). We recorded AFC, anti - Mullerian hormone (AMH), follicle stimulating hormone (FSH), AFC on day 3 of stimulation cycle (AFCbasal) and total number of oocytes collected. We used non -- parametric correlation and linear regression for statistical analysis.

Results: During the study period there were 23 women who fulfilled the study criteria. The median age was 37 (IQR 34 - 39). The median AMH was 6.4pmol/l (4.8 -- 9.0) , median FSH was 7.4iu/L (IQR 6.0 -- 9.5) and median (AFC) was 11 follicles (IQR 8 -- 13). The median AFCbasal was 9 (IQR 7 -- 12), median number of oocytes collected was 7 (IQR 5 -- 11). There was a significant positive correlation between number of oocytes collected and AMH ($r = 0.666$, $p=0.01$), AFC ($r = 0.605$, $p=0.001$) and AFCbasal ($r = 0.768$, $p= 0.001$). In a linear regression model AFCbasal was selected as the best predictor of number of oocytes collected.

Conclusion: We confirm that AFCbasal shows significant positive correlation with the eventual number of collected oocytes after ovarian stimulation. The potential of this parameter as a selection tool for stimulation cycles is to be evaluated.

1. Fauser CM. Intraovarian control of early folliculogenesis. 2. Decanter C, Robin G, Mailliez A, Sigala J, Morschhauser F, Ramdane N, et al. Prospective assessment of follicular growth and the oocyte cohort after ovarian stimulation for fertility preservation in 90 cancer patients versus 180 matched controls. Reprod Biomed Online. 2018;36(5):543-51.

SP2E.4 NLRP3 inflammasome activation in low ovarian reserve patients

Andrés Santé Carolina¹; Rancan Lisa²; Cordero Rosales Carolina¹; Rodrigo Carbajosa Andrea¹; Linillos Pradillo Beatriz²; Vara Ameigeiras Elena²; Tresguerres Jesús A.F.²; Cortés Gallego Susana¹

¹Clinica Tambre; ²Universidad Complutense de Madrid

Purpose/background/objectives: Chronic low-grade inflammation has emerged as a key contributor to the pathogenesis of several diseases that cause low ovarian reserve. The NLRP3 inflammasome is a multimeric protein complex that, through caspase activation, initiates an inflammatory form of cell death and triggers the release of proinflammatory cytokines like interleukin 1 beta (IL-1 β). It has been implicated in a wide range of diseases. However, a possible correlation of NLRP3 inflammasome activation and low ovarian reserve has not been described yet. Hence, this study aims to investigate it.

Methods: mRNA expression of NLRP3, IL-1 β , IL-1 receptor, caspase 1, caspase 12, caspase 18 and serpin were measured in oocyte-granulosa (CGs) complexes retrieved from 12 healthy fertile oocyte donors (<35 y.o. with at least a previous cycle with pregnancy) and compared with 12 low ovarian reserve patients (>35 y.o.) whom took part in a prospective observational study. Participants were stimulated with the same protocol (FSHr and triggering with GnRH analogues). The mRNA expression was measured by qRT-PCR and relative changes in gene expression were calculated using the 2- $\Delta\Delta$ CT method. No parametric tests were used to identify any significant difference between groups.

Results: The mRNA expression of NLRP3 was significantly increased in patients with low ovarian reserve compared to donors ($p=0.022$). Also caspase 1, 12 and 18 expressions were significantly increased in the patients group compared to donors ($p<0.0001$, $p=0.0008$ and $p=0.0003$ respectively). At the same, IL-1 β and its receptor were found increased in

the patients group compared to the donors one ($p=0.0003$ and $p=0.0001$ respectively).

On the other hand, the expression of serpin, an inhibitor of NLRP3 activation, was significantly lower in patients group compared to the donors ($p=0.0002$).

Conclusions: Our results show a higher activation of NLRP3 in patients with low ovarian reserve compared to donors suggesting a correlation between its activation and the pathogenesis of this process.

SP2E.5 The effect of Junctional zone Igf-2 deletion on the reproductive function of female offspring

Louca John; Yao Sijia; Lopez-Tello Jorge; Yong Hannah; Christoforou Efthimia; Sferruzzi-Perri Amanda

Cambridge University

Background: The importance of placental function in fetal development cannot be understated. It supplies nutrients and oxygen to the fetus and secretes hormones that mediate changes in maternal physiology to support fetal growth during pregnancy. Impaired placental function can lead to poor fetal growth, with long lasting impacts on health into adulthood [1], yet relatively little is known about the programming of the reproductive system of the offspring [2]. Previous work from our lab has shown altered folliculogenesis, a prolonged estrus phase and an increase in the estradiol: progesterone ratio in pups that had been supported by genetically-malfunctioning placentas (loss of Igf2 in the endocrine junctional zone of the placenta; Jz-Igf2 loss). The aim of this study was to determine whether these changes were accompanied with altered expression of genes involved in folliculogenesis and steroidogenesis, as well as defects in uterine morphology in Jz-Igf2 loss pups.

Methods: At 13 weeks, control and Jz-Igf2 loss offspring were sacrificed and ovaries and uteri weighed. Ovaries were then frozen for qPCR analysis or processed for histology. Data were analysed by t-test and significant when $p<0.05$.

Results: There were trends for altered steroidogenesis in the ovary as indicated by an increase in *Stard1* ($p=0.0564$) and *Pparg* expression ($p=0.053$) in the Jz-Igf2 loss females. However all other genes analysed were not altered (*Lhr*, *Fshr*, *Era*, *Erb*, *3bhsd*, *Hmgcr*, *Cyp17a1*, *Ppara*, *Leptin*, *Ki67* and *Caspase3*). There were also no changes in gross morphology of the uterus (proportion of perimetrium, myometrium, endometrium and lumen) and any difference in uterine abundance or distribution of actin, *Caspase-3* or *Ki67* in Jz-Igf2-loss, versus control female pups.

Conclusion: Changes in ovarian morphology and circulating sex steroid concentrations in female pups exposed to placental malfunction (in Jz-Igf2 loss) are accompanied with altered ovarian expression of steroidogenesis genes and normal uterine morphology.

1)Sferruzzi-Perri AN, Camm EJ. The programming power of the placenta. *Frontiers in physiology*. 2016 Mar 14;7:33.

2)Aiken CE, Tarry-Adkins JL, Ozanne SE. Transgenerational effects of maternal diet on metabolic and reproductive ageing. *Mammalian Genome*. 2016 Aug 1;27(7-8):430-9.

SP2E.6 Unravelling the naked mole rat ovary: a comparison between the queen and the worker

Clarkson Yvonne¹; McLaughlin Marie¹; Waterfall Martin¹; Holtze Susanne²; Boke Elvan³; Hildebrandt Thomas²; Telfer Evelyn¹

¹The University of Edinburgh; ²Leibniz Institute for Zoo & Wildlife Research; ³Centre for Genomic Regulation (CRG)

Background: The naked mole rat (NMR) (*Heterocephalus glaber*) is a burrowing rodent native to East Africa. NMR's have unique reproductive biology(1) and longevity(2). Within each NMR colony is a single ovulating female (queen), whose presence suppresses ovulation in all other females (workers, FW)(1). The aim of this study was to compare ovarian morphology between queen and FW NMRs and investigate the presence of putative oogonial stem cells (OSCs) using antibody-based fluorescence activated cell sorting (FACS). Previous studies(3, 4) have shown that putative OSCs can be isolated by FACS using an antibody to the germ cell marker DDX4.

Methods: Ovaries were collected from one queen and three FW NMRs. Tissue was 1) fixed for assessment of morphology using H and E or immunohistochemistry (using a DDX4 antibody) or 2) dissociated and immunostained with DDX4 for antibody-based FACS. Sorted cells were collected for RT-PCR.

Results: Queen and FW NMR ovaries contained primordial and developing follicles, however corpora lutea were only observed in the queen. Multiple closely-packed spherical structures (15-30 μ m) were observed in both tissue groups

and DDX4 staining confirmed these were naked oocytes/primordial follicles. FACS analysis confirmed the presence of DDX4-positive cells (4-12µm) in queen and FW cell suspensions. DDX4-positive cells identified in the queen were smaller and more complex in nature. Furthermore, there was a unique population identified in the queen that showed significantly higher intensity of DDX4. RT-PCR confirmed DDX4 gene expression in all sorted cells.

Conclusions: No difference was observed in the distribution of primordial and developing follicles between queen and FW ovaries. DDX4 protein and gene expression was confirmed in both groups. FACS analysis demonstrated a unique sub-population of DDX4 cells in the queen, which could represent putative OSCs. Further characterisation of this sub-population may determine its contribution to reproductive suppression and activation in NMR.

1. Margulis SW, Saltzman W, Abbott DH. Behavioral and hormonal changes in female naked mole-rats (*Heterocephalus glaber*) following removal of the breeding female from a colony. *Hormones and behavior*. 1995;29(2):227-47. 2. Buffenstein R, Jarvis JU. The naked mole rat—a new record for the oldest living rodent. *Science of aging knowledge environment* : SAGE KE. 2002;2002(21):pe7. 3. White YA, Woods DC, Takai Y, Ishihara O, Seki H, Tilly JL. Oocyte formation by mitotically active germ cells purified from ovaries of reproductive-age women. *Nature medicine*. 2012;18(3):413-21. 4. Clarkson YL, McLaughlin M, Waterfall M, Dunlop CE, Skehel PA, Anderson RA, et al. Initial characterisation of adult human ovarian cell populations isolated by DDX4 expression and aldehyde dehydrogenase activity. *Sci Rep*. 2018;8(1):6953.

SHORT PAPER SESSION 3A: OOCYTES, EMBRYOS AND IMPLANTATION FAILURE

SP3A.1 Live birth and perinatal outcomes in Recurrent Implantation Failure: an analysis of 1, 376,454 cycles from the Human Fertilisation and Embryology Authority database spanning 1991 to 2016

Smith William; Mascarenhas Mariano; Bhandari Harish

Leeds Fertility

Live Birth and Perinatal Outcomes in Recurrent Implantation Failure (RIF): An Analysis of 1,376,454 cycles from the Human Fertility and Embryology Authority (HFEA) database spanning 1991 to 2016. William Smith, Mariano Mascarenhas, Harish Bhandari Leeds Fertility, Leeds Teaching Hospitals NHS Trust, York Road, Seacroft, Leeds, LS14 4UH. Background: Multiple embryos are frequently transferred in women who have had RIF. This study aims to explore pregnancy outcomes and the number of embryos transferred in order to optimise future pregnancy outcomes for RIF women. Methods: From the HFEA anonymised register, we excluded cycles with donor gametes, surrogacy, pre-implantation genetic testing, frozen cycles and cycles in which embryo transfer (ET) was not performed. Live birth rate (LBR) and perinatal outcomes for singletons including preterm birth (sPTB) and low birth weight (sLBW) were analysed. Results We analysed 52,607 and 349,185 ETs for women with RIF and first-cycle ET (control arm). There were 7867 and 78740 singleton live births following RIF and first-cycle ET, respectively. The overall LBR per ET for RIF women was 20.90%, which was significantly lower (RR 0.71, 95% CI 0.70 to 0.72) than women having first-cycle ET (29.51%). The risk of sPTB was not significantly different (RR 1.07, 95% CI 0.99 to 1.15), nor was the risk of sLBW (RR 1.01, 95% CI 0.93 to 1.08). In the RIF group, 7058 had a single (SET), 30817 had two (DET) and 14729 had three (TET) embryo transfers and the singleton LBR was 13.64%, 16.38% and 12.61%, respectively. Achieving a singleton live birth at term (SLBT) was marginally better (RR 1.19, 95% CI 1.12 to 1.28) with DET (14.71%) compared to SET (12.33%), but carried a significantly higher risk of multiple pregnancy (RR 14.66, 95% CI 9.68 to 22.20). SLBT was significantly lower for TET compared to SET (RR 0.92, 95% CI 0.85 to

Human Fertility and Embryology Authority (HFEA). Anonymised register. Available from <https://www.hfea.gov.uk/about-us/our-data/> [Accessed 20 December 2018].

SP3A.2 The ovaries of transmen indicate effects of testosterone on the primordial follicle pool

Baillie Emily¹; McLaughlin Marie¹; Watson Neale²; Telfer Evelyn¹; Anderson Richard¹

¹University of Edinburgh; ²NHS

Background/objectives: There is a growing need for fertility preservation services for the transgender population, however, the effects of gender-affirming endocrine therapy on long term ovarian function are largely unknown. The objectives of this study are to investigate the effect of prolonged high dose testosterone therapy on the primordial follicle pool and small growing follicles.

Method: Human ovaries were collected from transgender men undergoing salpingo-oophorectomy after long-term treatment with testosterone, with biopsies of ovary from women collected at the time of caesarean section used as controls (n=12, aged 23-39). Fragments of ovarian cortex were prepared for tissue culture, and histological analysis was performed on samples at the time of collection and after culture.

Results: At day 0 (n=2, patients aged 25 and 36), analysis of 393 follicles showed there was a difference in ovarian follicle distribution. 94.1% of follicles were classified as non-growing in testosterone-exposed ovaries compared to 81.9% in control ovary ($p<0.05$). The proportion of morphologically healthy follicles was reduced in testosterone-exposed ovaries, with 56.0% of follicles being classified as healthy compared to 74.8% in controls ($p<0.05$). After 6 days of culture, analysis of 821 follicles showed the proportion of non-growing follicles declined significantly compared to day 0 in both groups, with a reciprocal rise in the percentage of primary and secondary follicles. At day 6, there was no significant difference in follicle distribution between testosterone-exposed ovaries and controls ($p=0.27$). However, there was a striking difference in follicle health, with only 24.6% follicles classified as normal from testosterone-exposed ovaries compared to 63.1% in controls ($p<0.05$).

Conclusion: The basis for and implications of the poor follicle health are unclear, but these data indicate that testosterone has previously unrecognised effects on the primordial and small growing follicles of the ovary.

SP3A.3 Do androgens and other reproductive hormones influence protein production in an endometrial cell line and therefore have an effect on embryonic implantation in relation to Polycystic Ovary Syndrome

Elliott Chloe; Marriott Andrew; Charnock Jayne

Edgehill University

Background: Polycystic Ovary Syndrome (PCOS) is a common endocrine disorder, with 5-10% prevalence of women of reproductive age. It is characterized by anovulation, hyperandrogenism and is associated with infertility. Research into infertility in PCOS has previously focused on anovulation, however implantation failure may also play a role, due to the occurrence of hyperandrogenism (1). Implantation following conception is facilitated by integrins such as $\alpha V\beta 3$, within the endometrial lining. $\alpha V\beta 3$ is necessary for implantation, along with cell adhesion and embryo-endometrial interactions. Previous research has shown that testosterone causes a decrease in HOXA10 expression, a gene responsible for the regulation of $\alpha V\beta 3$ (2).

Methods: Here the effects of testosterone were investigated in vitro on $\alpha V\beta 3$ expression and in a ligand-coated bead model of embryonic attachment. By culturing an endometrial adenocarcinoma cell line (Ishikawa), the rate of attachment of embryo-sized gel beads (coated in the $\alpha V\beta 3$ ligand, fibronectin) under different hormonal conditions was assessed. Different concentrations of testosterone were assessed within the model ($1 \times 10^{-8}M$ to $1 \times 10^{-4}M$) also.

Results: Attachment rates of beads were significantly decreased when Ishikawa cells were pre-treated with testosterone ($1 \times 10^{-4}M$) overnight ($p=0.0001$) prior to bead addition and attachment rates found to significantly decrease following treatment with $1 \times 10^{-6}M$ of testosterone ($p=0.0001$), indicating testosterone may cause disruption to the embryo attachment process in vivo. Pre-treatment of cells with progesterone ($1 \times 10^{-8}M$ to $1 \times 10^{-4}M$) showed a significant difference between bead attachment with untreated cells and cells in $1 \times 10^{-4}M$ progesterone ($p=0.0205$). Combined treatment of Ishikawa cells with both testosterone and progesterone prior to bead addition indicated that progesterone may rescue the effects of testosterone on $\alpha V\beta 3$ expression and therefore bead attachment.

Conclusion: These results suggest that testosterone causes downregulation $\alpha V\beta 3$, which may contribute to poor rates of implantation in women with PCOS and that progesterone may be considered as a candidate to oppose this action.

1. Daniel A. Dumesic et al, 2015: Scientific Statement on the Diagnostic Criteria, Epidemiology, Pathophysiology, and Molecular Genetics of Polycystic Ovary Syndrome, Endocrine Reviews 36 (5): 487-626
2. Dilek Cermik et al, 2003: Regulation of HOXA-10 Expression by Testosterone in Vitro and in the Endometrium of Patients with Polycystic Ovary Syndrome, The Journal of Clinical Endocrinology & Metabolism, Volume 88 (1): 238-243

SHORT PAPER SESSION 3B: ENVIRONMENTAL INFLUENCES ON METABOLIC AND REPRODUCTIVE HEALTH

SP3B.1 Male liver dysfunction as a consequence of prenatal androgen excess

Dryburgh Louise¹; Siemienowicz Kasia¹; Filis Pan²; Fowler Paul²; Duncan Colin³; Rae Mick¹; [Thomas Jennifer¹](#)

¹Edinburgh Napier; ²University of Aberdeen; ³University of Edinburgh

Introduction: Excess androgens are potential 'developmental disruptors' in utero, implicating them in the origins of Polycystic Ovary Syndrome (PCOS). A syndrome characterised by increased androgen concentrations in women, the male offspring from PCOS pregnancies are characterised by dyslipidaemia, implying altered hepatic function. Here we hypothesised that excess androgens can alter liver function in male offspring and utilised a direct testosterone application in developing ovine male fetuses for investigation. Given the critical nature of inflammation and fibrotic deposition in serious liver disease, we examined the expression of key components regulating these hepatic systems.

Methods: Ovine fetuses were directly injected with 200 µl testosterone propionate (PA; 20 mg) or vehicle control (C), under ultrasound guidance at day 62 and 82 of gestation. Male adolescent offspring were studied at 6 months postnatal age (C, n=14; PA, n=14). Hepatic transcriptome was defined by Illumina RNA sequencing and findings confirmed by qPCR. Statistical analysis between C and PA groups was performed using pairwise comparisons, with false discovery rate correction, accepting P<0.05 as significant. Hepatic collagen deposition was determined by Sirius Red staining of histological sections.

Results: After fetal exposure to excess androgens, mRNA expression of key chemokine receptors and ligands CXCR6, CXCR3, CXCR2, CXCL1, CXCL9, CXCL14, CXCL11, CCL5 and also TIMP1, IL10, ARG1 were significantly decreased in male adolescent livers. Significant increases in five isoforms of Collagen IV, Collagen alpha-1(XVIII), LAMB1 and LAMB3, MMP2, VEGFA, SMO and ADAM12 were observed. The critical enzyme system which drives collagen deposition, TGFβ-1, CTGF and LOX were down regulated in response to prenatal androgen excess, providing explanation of why we did not observe increased fibrosis at this stage of postnatal life.

Conclusions: Excess prenatal androgenic exposure in developing male fetuses leaves a legacy of dysregulated immune and fibrotic systems which has implications for lifelong liver health and disease.

SP3B.2 Placenta endocrine malfunction programmes adult offspring metabolic health in a sex-dependent fashion

Christoforou Efthimia; Lopez-Tello Jorge; Yong Hannah; Sandovici Ionel; Forhead Alison; Constancia Miguel; [Sferruzzi-Perri Amanda](#)

University of Cambridge

Background/Aims: Perturbations in the endocrine and nutritional in utero environment programme changes in the metabolic health of offspring postnatally. However, little is known about the placenta, an organ involved in the regulation of the endocrine and nutritional environment of the fetus, in the metabolic health of adult offspring. We thus aimed to assess the effect of genetically-induced overgrowth of the mouse placental endocrine zone (junctional zone, Jz) on offspring metabolic health.

Method: Placental Jz overgrowth was achieved through conditional disruption of imprinting of the H19-Igf2 locus, which results in Jz expansion (Jz-ICR1Δ). Litters were delivered naturally, standardised to 3 female and 3 male pups, and after weaning (at three weeks of age), were fed either a chow or obesogenic diet. At 16 weeks of age one pup per sex, per litter received either an insulin or glucose tolerance test. The liver and pancreas were collected to perform western blotting of the PI3K pathway and ELISAs for insulin content respectively. Findings were compared to control offspring that have a normal H19-Igf2 imprinting and unaltered Jz size.

Results: On a chow diet, male Jz-ICR1Δ offspring were less sensitive to insulin and had a decreased pancreatic insulin content compared to controls, whilst Jz-ICR1Δ female offspring had unaltered insulin sensitivity. On an obesogenic diet, male Jz-ICR1Δ offspring showed greater insulin resistance, whereas female Jz-ICR1Δ offspring were more tolerant to glucose than their respective dietary controls. The hepatic abundance of proteins involved in glucose and insulin

handling were altered in Jz-ICR1Δ offspring on both a chow and an obesogenic diet, although the specific nature of these changes depended on offspring sex.

Conclusions: Placental endocrine malfunction programmes changes in adult offspring metabolic health in a sex-dependent manner. The molecular mechanisms underlying the sexually dimorphic programming of the offspring are currently being further explored.

SP3B.3 Assessing the impact of paternal diet on testicular morphology and gene expression in mice

Eid Nader¹; Morgan Hannah L.¹; Gavriel Christiana¹; Castellanos Uribe Marcos²; Khan Iqbal²; Watkins Adam J.¹

¹Division of Child Health, Obstetrics and Gynaecology, School of Medicine, Queen's Medical Centre, University of Nottingham; ²Nottingham Arabidopsis Stock Centre (NASC), School of Biosciences, Sutton Bonington, University of Nottingham

Background: There is growing evidence that poor paternal diet adversely affects sperm quality, which consequently impacts on embryonic development and offspring health.

Methods: To better understand the impact of diet on male testicular and reproductive fitness, we fed C57BL/6 male mice either a control normal protein diet (18% casein; NPD), isocaloric low protein diet (9% casein; LPD), a low protein diet supplemented with methyl donors (betaine, choline chloride, folic acid, methionine, vitamin B12; MD-LPD), a high fat Western diet (24% fat; WD), or a Western diet supplemented with methyl donors (MD-WD) for ≥7 weeks. Testes were collected and processed for either morphological assessment (histology) or gene expression (microarray) analysis.

Results: No significant differences were observed in the stud male weights between treatment groups at the time of sacrifice. Furthermore, no significant differences in mean seminiferous tubule cross-section area, perimeter, luminal area, or epithelial area were observed between the five groups. However, abnormalities such as tubular epithelial loss, tubular vacuolisation and basal membrane separation were observed more frequently in testes exposed to WD and MD-WD compared to the other study groups. Microarray analysis revealed that the greatest change in gene expression relative to NPD was in the WD group, where 134 genes were up-regulated while 269 genes were down-regulated (fold change = 1.1, +FDR, p=0.05). Preliminary pathway analysis suggests the involvement of processes such as gametogenesis, abnormal testis morphology, abnormal embryo development, embryo lethality, abnormal mitochondrial physiology, triglyceride regulation, and calcium regulation.

Conclusions: These data provide further insight into testicular morphology and global gene expression patterns in response to poor paternal diet with and without key vitamin and mineral supplementation and the possible underlying mechanisms taking place. Ongoing research aims to validate gene expression profiles and investigate underlying molecular pathways to further elucidate the impact of diet on male reproductive fitness.

SP3B.4 Effect of environment and diet on semen quality in man and canines

Gardner David¹; Tomlinson Matt²; Pooley Karen²; Bastidas Mary¹; England Gary¹; Richards James³; Lea Richard¹

¹University of Nottingham; ²Nottingham University Hospitals NHS Trust; ³University of Birmingham

Objectives: Recent evidence collated from westernised, industrial countries suggests human sperm counts have declined ~50% from 1980 to 2015. A similar temporal decline has been noted in assistance canines living in UK households. This study compared physiology of live sperm, seminal fluid composition and sperm morphology in humans and canines that have never lived in a modern, westernised household environment versus those that have always lived in such an environment.

Methods: Fresh semen was collected from humans and canines and immediately analysed using an iCASA system for sperm physiology. Biobanked semen was later analysed for trace element profile by ICP-MS and presence of environmental contaminants. Ethical approval for healthy volunteers and for canine work was obtained from ethical review committees at the university of Nottingham (REC code: 194-1901 & 2202-180206, respectively). Three different groups of men or dogs (n=10-18/group) were sampled at source in varying locations differing by habitual household environment. Accompanying differences in diet (i.e. predominantly refined vs. unrefined) were recorded, as appropriate using diet diaries.

Results: Sperm physiology and seminal fluid composition were broadly similar between humans and canines that had never, versus those that had always, lived in a modern, westernised household environment. Secondary effects of diet on semen quality were marked in canines -- that had extreme diet differences -- but were minimal in humans, where diet differences were only notable by consumption of an un-refined vs refined diet. Multi-variable modelling of seminal fluid trace element composition successfully discriminated UK from Andean from Amazonas semen, suggesting either a geographical or an ethnic contribution to semen quality.

Conclusion: Traditional living has no overt beneficial effect on semen quality. Extremes of diet can influence parameters of semen quality, but this is unlikely for humans. Semen could be discriminated by geographical location, suggesting a subtle influence of environment.

SHORT PAPER SESSION 3C: EMBRYONIC DEVELOPMENT AND METABOLISM

SP3C.1 Changes in metabolic profiles of blood plasma and blastocyst cavity fluid in response to maternal diabetes during early pregnancy

Schindler Maria; Pendzialek Sophia; Grybel Katarzyna; Seeling Tom; Navarrete Santos Anne

Institute of Anatomy and Cell Biology

Objectives: Changes in metabolite concentrations are suitable indicators of metabolic activity during preimplantation embryo development. During this period the embryo develops from a metabolically inert stage at ovulation to a rapidly metabolising blastocyst at implantation. The goal of this study was to interrogate biochemical profiles manifested in rabbit blastocyst cavity fluid (BF) and plasma collected from animals with and without diabetes mellitus type 1.

Methods: The insulin-dependent diabetes was induced chemically by alloxan in female rabbits before pregnancy. On day 6 post coitum maternal plasma and BF were collected for metabolic profiling by Ultrahigh Performance Liquid Chromatography-Tandem Mass Spectroscopy (UPLC-MS/MS) (Metabolon Inc. Durham, NC, USA).

Results: Datasets comprised a total of 284 compounds of known identity in BF and 597 in plasma. As predicted, glucose, fatty acids and their derivate were elevated in plasma of the diabetic rabbits. Interestingly, the BF fluid did not show an increase in glucose. Another notable difference between plasma and BF involved additional carbohydrate pathways. In plasma, the sorbitol, mannose, and pentose pathways were elevated in the diabetic group, which may be a means of dealing with excess glucose. In BF, the only other carbohydrate pathway that was altered was pentose metabolism. Other changes were more consistent between BCF and plasma. Both displayed elevated acylcarnitines, BHBA, and multiple compounds within the BCAA metabolism pathway suggesting elevated levels of lipid beta-oxidation in the diabetic group.

Conclusion: Our results indicate a distinct metabolic signature in maternal plasma and BF due to a diabetes mellitus and advance understanding of the pathology of periconceptional diabetes. This work was supported by the German Research Council (DFG ProMoAge, GRK 2155) and the EU (Epihealth, EpihealthNET).

SP3C.2 Aneuploidy screening in inner-cell mass and trophectoderm lineages of bovine blastocysts derived from stimulated and non-stimulated ovarian cycles

Tutt Desmond A R¹; Silvestri Giuseppe²; Simmons Robert³; Kwong Wing-Yee¹; Guven-Ates Gizem¹; Clare Constance E¹; Blondin Patrick⁴; Handyside Alan H⁵; Griffin Darren K²; Sinclair Kevin D¹

¹School of Biosciences, University of Nottingham, UK; ²School of Biosciences, University of Kent, UK; ³Paragon Veterinary Group, UK; ⁴L'Alliance Boviteq Inc, Canada; ⁵London Women's Clinic, London, and the School of Biosciences, University of Kent, UK

Background: Aneuploidy is a major factor associated with pregnancy failure in human assisted reproduction and is observed in in vitro produced bovine embryos [1,2]. Screening of bovine trophectoderm biopsies can identify aneuploidy [2], and this is associated with pregnancy outcomes following embryo transfer [3]. Chromosomal mosaicism, however, is prevalent in human embryos [4], questioning the reliability of aneuploidy screening in trophectoderm biopsies. We evaluated aneuploidy in the inner-cell mass (ICM) and trophectoderm of bovine blastocysts from either stimulated or non-stimulated cycles.

Methods: Nine sexually-mature virgin heifers underwent successive cycles of transvaginal follicular aspiration with (n=3 cycles) or without (n=6 cycles) hormone stimulation as described previously [2]. Oocytes meeting strict-grading criteria were matured, fertilised and cultured to Day 8. Nine blastocysts per donor per treatment (i.e. +/- stimulation) meeting criteria [5] for transfer were immuno-dissected into ICM and trophectoderm lineages. These lineages (from single embryos) underwent aneuploidy screening [2,3]. Proportions were analysed by logistic regression assuming binomial errors. Remaining data was analysed by ANOVA.

Results: Developmental rates were high overall, but lower for non-stimulated than stimulated cycles with fewer oocytes matured per donor cycle (6.7 ± 0.35 vs 18.6 ± 0.91 ; $P < 0.001$), lower proportions cleaved of inseminated (0.89 ± 0.020 vs 0.95 ± 0.012 ; $P = 0.013$) and blastocysts of cleaved (0.53 ± 0.037 vs 0.75 ± 0.027 ; $P < 0.001$). Proportionally fewer blastocysts were of transferrable quality (0.78 ± 0.041 vs 0.88 ± 0.024 ; $P = 0.039$). Initial analyses indicate overall incidence of aneuploidy was low (0.11), but higher in blastocysts from non-stimulated than stimulated cycles (0.22 vs 0.00). Aneuploidy was more prominent in Grade 2 (0.33) than Grade 1 (0.04) blastocysts. Triploidy of maternal origin was the most common anomaly. There was only limited evidence of discordance between trophectoderm and ICM lineages.

Conclusion: Current protocols lead to high yields of transferrable blastocysts (~11 per stimulated cycle), possibly due to low incidence of aneuploidy. Confirmation awaits completion of ongoing analyses. BBSRC-LINK (BB/R007985/1).

1. Fragouli E and Wells D. Aneuploidy in the human blastocyst. *Cytogenetic and Genome Research*. 2011; 133:149-59.
2. Turner KJ, Silvestri G, Black DH, Dobson G, Smith C, Handyside AH, et al. Karyomapping for simultaneous genomic evaluation and aneuploidy screening of preimplantation bovine embryos: The first live-born calves. *Theriogenology*. 2018; 125: 249-58.
3. Serrano-Albal M, Silvestri G, Vining LM, Labrecque R, Marras G, Larmer S, et al. SNP based Preimplantation Genetic Testing for Aneuploidy (PGT-A) to improve pregnancy outcomes in cattle IVP: a blind retrospective study. *Animal Reproduction*. 2019; 16: 757.
4. Taylor TH, Gitlin SA, Patrick JL, Crain JL, Wilson JM, Griffin DK. The origin, mechanisms, incidence and clinical consequences of chromosomal mosaicism in humans. *Human Reproduction Update*. 2014; 20: 571-81.
5. Stringfellow DA, Seidel SM. *Manual of the International Embryo Transfer Society*. IETS, Savoy, IL1998. p. 103-30.

SP3C.3 Minimising miscarriage in ART: IVF or ICSI-derived euploid blastocysts?

Enakshi Ali Zoya¹; Ahmed Odia Rabia²; Seshadri Srividia²; Sen Gupta Sioban¹; Cawood Suzanne²; Serhal Paul²; Naja Roy³; Viñals Gonzalez Xavier²

¹University College London; ²The Centre for Reproductive and Genetic Health; ³Igenomix

Background: Aneuploidy is the leading cause of implantation failure, miscarriage and congenital abnormalities in humans. PGT-A ought to enhance clinical outcomes by improving implantation rates and reducing time to pregnancy, particularly for patients with increased risk of embryo aneuploidy. The primary objective of the study includes analysing the effects of insemination technique on embryonic ploidy status and to analyse the clinical outcomes following single euploid embryo transfer between the IVF and ICSI cohort.

Methods: A total of 1315 blastocysts from 309 PGT-A cycles were analysed, including 102 IVF and 207 ICSI cycles, generating 442 and 873 biopsied blastocysts, respectively.

Results: Pre-treatment variables for both cohorts were matched and there were non-significant differences between the ploidy status between the cohorts: euploidy-rate (32.3% IVF v/s 34.2% ICSI, $P = 0.15$); aneuploidy-rate (64.7% IVF v/s 69% ICSI, $P = 0.099$), mosaicism-rate (3% IVF v/s 4.9% ICSI, $P = 0.115$). Despite there being non-significant differences between the IVF and ICSI cohorts in the implantation rate (62.3% v/s 56.5%, $p = 0.454$) and clinical pregnancy rates (53.5% v/s 50.7%, $p = 0.408$), there was a significant difference in the pregnancy outcomes, such that the ICSI cohort had a lower miscarriage rate and higher ongoing pregnancy rate (47.8% ICSI v/s 37.7% IVF, $p = 0.0018$) compared to the IVF cohort. A subsequent regression analysis showed that the odds of miscarriage were marginally lower in the ICSI cohort by a factor of 1.15 (CI 0.4-0.7, $p = 0.048$), in other words, insemination technique could impact the odds of miscarriage by up to 15%.

Conclusion: Considering the ploidy rates in both cohorts are not different, the use of IVF could be justified over ICSI; however, owing to the subgroup analysis indicating a higher miscarriage rate, our findings suggest against the use of IVF for patients with non-male factor undergoing PGT-A.

SP3C.4 Hyaluronidase-2 changes morphokinetics of in vitro cultured bovine embryos

Fouladi-Nashta Ali¹; Bacon Katy¹; Hartshorne Geraldine²

¹Royal Veterinary College; ²University of Warwick and University Hospitals Coventry and Warwickshire NHS Trust

We have previously shown size-specific effects of hyaluronan (HA) on embryo development and the beneficial effects of hyaluronidase-2 (hyal-2) on improving blastocyst formation and quality in bovine. This effect is mediated through small HA fragments of 20 kDa binding to HA receptors CD44 and RHAMM, present in bovine embryos. In the present study, we have analysed the impact of hyal2 on the morphokinetics of cell division in bovine embryos. Embryos were produced in four independent repeats after in vitro maturation and in vitro fertilisation of abattoir-derived oocytes. Twenty-four hours after IVF, presumptive zygotes or cleaved 2-cell embryos (n= 96) were cultured in the absence or presence of 300IU/ml hyal-2 in BO-IVC medium (IVF Bioscience) in an Embryoscope incubator. Times taken for the embryos to reach 4-cell, 8-cell, morula and blastocyst stages and hatched blastocyst were recorded, and the cell numbers in blastocysts were counted after Hoechst staining at 186h hours post fertilisation. The average time (hours post fertilisation; HPF) for the embryos reach to 4-cell stage in the control medium was longer (43.1 ± 0.6) than for embryos treated with hyal-2 (36.4 ± 0.5 , $P < 0.0001$). Similarly, hyal-2 treated embryos arrived faster at 8-cell (47.8 ± 0.5 v. 63.9 ± 1.2 ; $P < 0.0001$), morula (92.1 ± 0.6 v. 108.1 ± 1.0 ; $P < 0.0001$), blastocyst (158.2 ± 1.4 v. 174.1 ± 1.6 ; $P < 0.0001$) and hatched blastocyst (177.7 v. 190.4 HPF $p < 0.05$) stages. However, no statistical difference was found in the total cell number between hyal-2 treated (91.5 ± 13.5) and control (77 ± 12.9) embryos. The findings of this study provide significant results showing the potential use of hyal-2 as a culture media supplement for bovine embryos. Further studies are required to explore the role of HA signalling in cell division in embryos and to test the effects in other species.

SHORT PAPER SESSION 3D: REPRODUCTIVE ENDOCRINOLOGY

SP3D.1 Immunological stress during development might delay pubertal onset through altering Srd5a1 gene regulation in GnRH-neurons

Keestra Sarai¹; Bar Sade Ben²; Bentley Gillian³; Stöger Reinhard⁴; Melamed Philippa²

¹School of Anthropology, University of Oxford; ²Faculty of Biology, Technion-Israel Institute of Technology; ³Department of Anthropology, Durham University; ⁴Faculty of Science, Nottingham University

Background: Using a GnRH-neuron cell culture and life-history perspective, we examined the role of immunological stress in reproductive development. We have previously shown that early life conditions influence pubertal progression and progesterone levels in Bangladeshi migrants to the UK arriving before age eight, when adrenarcheal development and dehydroepiandrosterone (DHEA) production occur(1). A mouse-model of early life inflammation confirmed that immunological stress delays puberty(2). The gene Srd5a1 (encoding the enzyme 5 α -reductase which catalyses neurosteroid synthesis) was more methylated in Bangladeshi women, and downregulated the hypothalamus of immune-stressed mice(2). We hypothesised that immunological stress alters Srd5a1 gene regulation, causing lower 5 α -reductase and neurosteroid levels. This would decrease positive GABAA-receptor modulation, reducing GnRH-neuron excitability and delaying puberty.

Methods: Mouse GnRH-neurons (GT1-7) were treated with various concentrations of DHEA, oestradiol, progesterone, dihydrotestosterone (DHT), or dexamethasone, mimicking hormonal changes during adrenarche, puberty, and stress. Gene expression was measured using quantitative RT-PCR. A ChIP-experiment was conducted to locate glucocorticoid receptor-binding sites in the Srd5a1 promoter. GnRH-secretion into culture medium was measured by ELISA after exposure to progesterone, a 5 α -reductase-blocker, dutasteride, and/or a GABAA-receptor agonist, muscimol.

Results: DHEA and oestradiol upregulated Srd5a1 expression, but progesterone and DHT did not; dexamethasone downregulated Srd5a1 mRNA-levels. DHEA could offset dexamethasone-induced Srd5a1 downregulation. However, the glucocorticoid receptor did not appear to bind the mouse Srd5a1 gene's proximal promoter. Muscimol increased GnRH-secretion, which was augmented by progesterone's presence, but dutasteride prevented this effect.

Conclusion: Srd5a1 could influence pubertal onset by enhancing positive neurosteroid modulation of GABAA-receptors in GnRH-neurons. Our findings suggest that immunological stress during pre-pubertal development could inhibit Srd5a1 upregulation by DHEA, potentially explaining delayed puberty in Bangladeshi girls. These preliminary data

support observations of a developmental window at adrenarche when immunological stress could affect neurosteroid metabolism and fine-tune the GABAergic system. These findings could explain plasticity in later pubertal timing.

1. Núñez-de la Mora, A., Chatterton, R. T., Choudhury, O. A., Napolitano, D. A., & Bentley, G. R. (2007). Childhood Conditions Influence Adult Progesterone Levels. *PLoS Medicine*, 4(5), e167. <https://doi.org/10.1371/journal.pmed.0040167>
2. Bar Sade, B., Eden, O., Pnueli, L., Begum, K., Leeman, G., Emes, R. D., . Melamed, P. (Unpublished). Epigenetics explains plasticity of human reproductive function.

SP3D.2 Plasma profile of follicle-stimulating hormone after a single epidural administration via caudal vertebrae in cattle

Sakaguchi Kenichiro¹; Suda Tomoko²; Nattapong Ninpetch³; Yanagawa Yojiro³; Katagiri Seiji³; Yoshioka Koji²; Nagano Masashi⁴

¹University of Edinburgh; ²National Institute of Animal Health, National Agriculture and Food Research Organization; ³Hokkaido University; ⁴Kitasato University

Background: Conventional bovine superovulation regimens involve multiple intramuscular follicle-stimulating hormone (FSH) injections; these are stressful for animals and onerous. A single epidural FSH administration via the caudal vertebrae also induces superovulation in Japanese black cows (1). Therefore, we investigated peripheral FSH concentrations after a single epidural FSH administration in non-milking dairy cattle.

Methodology: Non-milking Holstein cows (n=3) received twice-daily porcine pituitary FSH (pFSH-P) (total 30 armour units) intramuscularly for 3 days (control). Two to four months later the same animals received a single epidural administration of pFSH-P (30 armour units) (epidural). Blood was sampled intensively from 0 to 104 hours after first pFSH-P administration. Plasma FSH concentrations were measured by the time-resolved immunofluorometric assay, using anti-pFSH serum as a primary antibody and pFSH as a reference standard, to compare between control and epidural treatments at each time point and compared to basal concentrations (0 hours). The numbers of large follicles (≥ 10 mm in diameter) at estrus and corpora lutea at luteal phase (Days 7, 10, or 18) were counted.

Results: The numbers of large follicles (control: 18.3 ± 7.5 , epidural: 15.7 ± 4.0) (mean $\pm$ SD) and corpora lutea (control: 7.3 ± 4.2 , epidural: 8.0 ± 2.6) were similar in both treatments. Plasma FSH concentrations in both treatments increased from 3 hours post-administration and were higher than 0 hours onwards in all animals. The peak levels of FSH were observed at 7.7 ± 4.5 hours in control and 5.7 ± 2.5 hours in epidural group after administration. Plasma FSH concentration was higher in epidural treatment compared to control ($p < 0.01$).

Conclusion: Results indicate that plasma FSH concentrations were maintained at higher level after a single epidural administration for more than 4 days. Superovulation efficiency was similar after both treatments in non-milking Holstein dairy cows.

1. Sakaguchi K, Ideta A, Yanagawa Y, Nagano M, Katagiri S, Konishi M. Effect of a single epidural administration of follicle-stimulating hormone via caudal vertebrae on superstimulation for in vivo and in vitro embryo production in Japanese black cows. *J Reprod Dev*. 2018;64(5):451-5.

SP3D.3 The effects of FSHR positive allosteric modulator on FSH dependent FSHR homomerisation and signal pathway activation

Agwuegbo Uche¹; Bousfield George²; Albert Anthony³; Jonas Kim¹

¹King's College London; ²Wichita State University; ³St George's University of London

The glycoprotein hormone, follicle stimulating hormone (FSH), and its target G protein-coupled receptor (FSHR), are essential for reproduction. In females, they play an important role in folliculogenesis and are key therapeutic drug targets for assisted reproductive techniques such as IVF, therefore understanding what modulates their function remains paramount. Post-translational modification of FSH gives rise to two predominant glycoforms; partially glycosylated FSH (FSH21), displays faster binding kinetics to the FSHR, more potent at activating cAMP-dependent signal pathways and more abundant in women of reproductive prime, in contrast to fully glycosylated FSH (FSH24), which is less bioactive and more abundant in menopausal women. Many GPCRs, including FSHR, have been shown to self-associate and form homomers, which is an important mode of regulating GPCR function. Our unpublished data suggests that higher bioactivity of FSH21 is mediated through time-dependent dissociation of FSHR oligomers into lower order homomers and monomers. However, little effect of FSH24 on FSHR homomers and monomers was

observed. Since FSH24 displays slower binding kinetics to the FSHR, we aimed to determine the effect of a FSHR positive allosteric modulator, Compound 2 (C2), on FSH24 binding, FSHR homomerisation and signal pathway activation. Super-resolution imaging via PD-PALM of HEK293 cells transiently expressing HA-FSHR revealed 30-minute pre-treatment $\pm 1\mu\text{M}$ C2 had no effect on the total number, nor the type of FSHR monomers and homomers observed at the plasma membrane at both 2- and 5-minute treatments with FSH24. Interestingly, Western blot analysis revealed that C2 pre-treatment with FSH24 induced no further changes in CREB phosphorylation until 15-minute treatments, where an enhancement was observed. These data suggest that C2 may be a powerful tool for regulating FSH/FSHR signal strength with potential as a novel therapeutic strategy for enhancing the efficacy and kinetics of endogenous FSH during ovarian stimulation protocols of IVF in aging women.

SP3D.4 Effects of Grape Seed Extracts on reproductive parameters in broiler breeder hens

Barbe Alix¹; Mellouk Namy²; Rame Christelle¹; Grandhayé Jeremy¹; Staub Christophe³; Cirot Marine³; Venturi Eric³; Petit Angélique⁴; Anger Karine⁵; Chahnamian Marine⁵; Rat Christophe⁵; Delaveau Joel⁵; Mairel François⁶; Riva Antonella⁶; Froment Pascal¹; Dupont Joelle¹

¹INRA UMR85 Unité de Physiologie de la Reproduction et des Comportements PRC; ²National Institutes of Health; ³INRA UE1297 Physiologie Animale de l'Orfraise; ⁴INRA UMR Biologie des Oiseaux et Aviculture; ⁵INRA UE Pole d'Experimentation Avicole de Tours; ⁶INDENA

The breeding hens fed ad libitum and selected for rapid growth, exhibit excessive fattening leading to fertility problems including multiple ovulations. To avoid these deleterious effects, a food restriction is practiced in breeding. However, this food practice is a source of stress. To reduce fattening and oxidative stress, grape seed extracts (GSE) rich in polyphenols were considered in our study as a dietary supplement. Indeed, we previously demonstrated that GSE, increased progesterone production in both human and hen primary granulosa cells. Here, we investigated the in vivo effects on the fertility parameters, egg quality and the progeny.

Four groups with 80 animals were monitored from 23 weeks to 39 weeks: control (A), supplemented since the growth period (4 week-old) with 0.5% (B) and 1% (C) of GSE and supplemented with 1% since the birth (D). We assessed the fertility, the egg parameters at 26 weeks (by Instron 5543, UK527), and the steroids and androgen production in yolk by ELISA assay. We also determined the mortality level and the growth of offsprings.

In plasma, at 40 weeks, the oxidative stress index was decreased for all GSE supplemented groups. At 26 week, the length and the width of the eggs were higher for the supplemented groups compared to the control and the eggshell was increased for the B and C groups. We showed a decrease in progesterone, androstenedione and testosterone concentration in yolk but there was no effect on the embryonic mortality and fertility parameters. However, we observed a significant increase in the viability and the body weight of the offsprings that could be explained by a reduction in the testosterone level in yolk. Taken together, GSE food supplementation did not affect in vivo hen fertility but it significantly decreased the mortality of offsprings.

POSTER PRESENTATIONS

P001 The union of robotics and deep learning for fully automated sperm assessment

Thomas Daniel¹; Maillot Robin¹; Bakalova Daniela²; Monteiro Miguel¹; Damart Tanguy¹; Homa Sheryl²; Taha Mohamed¹

¹Mojo Diagnostics, France; ²University of Kent

Background: While the assessment of sperm concentration, motility, and morphology has been well established as a front line screening for male infertility, its relevance in determining diagnosis is heavily reliant on technique and sampling¹. To combat variability, the WHO provides detailed instructions calculated to ensure proper sampling. However, these processes take a great deal of training to perform².

Computer-Assisted-Sperm-Analysis (CASA) tools have come to prominence with the potential to solve these issues while even providing new information³. Despite these value propositions, computer vision systems face several limitations. The assessment of concentration and motility is made difficult due to the low contrast of cells, frequent occlusions, and the large variability in debris types that can be found in human semen⁴.

Due to their ability to learn from vast amounts of data, neural networks have shown great promise as tools for object identification and classification, especially for medical applications^{5,6}.

Objective: We present a fully automated robotic microscope running a deep learning algorithm for hands-free semen analysis. This device is designed to follow the WHO protocols to maintain routine lab processes. It automatically captures high-speed, high-resolution images taken on standard coverslip preparations and uses a Convolutional Neural Network (CNN) to classify sperm cells, debris, and other cells.

Methods: The custom CNN was trained using 1000 images from 72 consenting routine patients with ethics committee approval at The Doctor's Laboratory in London.

Results: This device was able to classify sperm cells correctly 98% of the time. Furthermore, a Spearman correlation of 0.968 was obtained with an R² value of 0.88 for sperm concentration compared with routine manual analysis results. Lastly, it correctly evaluated oligozoospermia in 97% of patients.

Conclusion: Digitizing clinical analysis of semen has the potential to provide reliable and novel information for diagnosis. Through robotics and deep learning, clinically actionable results can be achieved.

1. Bjorndahl L. What is normal semen quality? On the use and abuse of reference limits for the interpretation of semen analysis results. *Human Fertility*. 2011;14(3):179-186.
2. Organization W. WHO Laboratory Manual for the Examination and Processing of Human Semen. Geneva: World Health Organization; 2010.
3. Alquezar-Baeta C, Gimeno-Martos S, Miguel-Jimenez S, Santolaria P, Yaniz J, Palacin I et al. OpenCASA: A new open-source and scalable tool for sperm quality analysis. *PLOS Computational Biology*. 2019;15(1):e1006691.
4. Yeste M, Bonet S, Rodriguez-Gil J, Rivera Del Alamo M. Evaluation of sperm motility with CASA-Mot: which factors may influence our measurements?. *Reproduction, Fertility and Development*. 2018;30(6):789.
5. Nissen M, Krause O, Almstrup K, Kjaerulff S, Nielsen T, Nielsen M. Convolutional Neural Networks for Segmentation and Object Detection of Human Semen. *Image Analysis*. 2017;:397-406.
6. Thirumalaraju, P., Bormann, C., Kanakasabapathy, M., Doshi, F., Souter, I., Dimitriadis, I. and Shafiee, H. (2018). Automated sperm morphology testing using artificial intelligence. *Fertility and Sterility*, 110(4), p.e432.

P002 Success of surgical sperm retrieval in men with cystic fibrosis

Lacey Lauren¹; Wilkinson Joanna²; Hassan Sibte³; Hunter Helen³; Bright-Thomas Rowland J⁴; Akhtar Muhammad A³; Heck Andrew⁵

¹University of Warwick; ²University of Manchester; ³Old Saint Mary's Hospital, Manchester University NHS Foundation Trust; ⁴Manchester Adult Cystic Fibrosis Centre, Manchester University NHS Foundation Trust; ⁵Manchester University NHS Foundation Trust

Background: Congenital bilateral absence of the vas deferens (CBAVD) is found in 1-2% of men with infertility and affects 95% of men with cystic fibrosis (CF). With surgical sperm retrieval (SSR) procedures including percutaneous epididymal sperm aspiration (PESA) and testicular sperm extraction (TESE) and subsequent intracytoplasmic sperm

injection (ICSI), men with cystic fibrosis can become biological fathers. Our aims are to report outcomes of SSR and pregnancy outcomes of SSR-ICSI in couples where the male partner has cystic fibrosis.

Methods: Retrospective cohort study over 8 years (2012-2019) at an NHS teaching hospital of 19 men with CF who had SSR and 15 couples who subsequently had ICSI. Data were collected using hospital computer systems/patient notes.

Results: The mean age and standard deviation of males and females in the cohort was 29.5 +/-4.7 years and 29.2 +/- 4.8 years respectively. The mean and standard deviation of preoperative FSH and testosterone levels for males was 4.9 +/- 2.6 IU/L and 13.6 +/- 3.7nmol/L respectively. The median and interquartile range of preoperative LH for males was 4.4 (3.3-6.9) IU/L. Three males had a testicular size of less than 15ml. All men had normal karyotyping. 15 couples have used sperm in one ICSI cycle. The live birth rate per cycle was 60% and the live birth rate per embryo transferred (PET) was 42.8%. No postoperative complications were reported in males following SSR.

Conclusions: Our study demonstrates a very positive outlook for men with azoospermia associated with CF. Although our numbers are small, when compared to the overall live birth rate PET rate reported by the HFEA in 2017 of 22% (1), the live birth rate PET in our cohort is approximately double. Couples can therefore be counselled about the high chances of achieving a live birth using ICSI following SSR.

1. Human Fertilisation & Embryo Authority. Fertility treatment 2017: Trends & Figures. HFEA; 2019.

P003 High-throughput phenotypic screening using human spermatozoa for the identification of novel compounds which improve sperm motility

Johnston Zoe; Gruber Franz; Andrews Paul; Barratt Christopher

University of Dundee

Background: Treatment options for men with poor sperm motility is limited to IVF or the use of certain vitamins and antioxidants, for which there is limited evidence of efficacy. Targeting the motility of sperm directly may be an effective and non-invasive avenue to pursue in fertility drug discovery. However, as sperm are highly specialised cells that do not divide, transcribe or translate, they are potentially difficult to target. Additionally, due to the differences between fertilization in humans and animal models it is essential human spermatozoa are used.

Aim: To employ a high throughput screening system measuring sperm motility to identify novel compounds with potential therapeutic utility.

Methods: Our lab has successfully developed a high-throughput phenotypic screening platform to assess the motility of human sperm using image based kinetic analysis. Screening collections of approved drugs and compounds at various stages of pre-clinical and clinical development, chemical probes, and other bioactives opens the potential of repurposing drugs and discovering novel medicinal chemistry start points as well as new sperm biology.

Results & Conclusions: The novel screening platform creates the opportunity to repurpose drugs, discover new pre-clinical candidates, and reveal new aspects of sperm biology. We have screened over 15,000 compounds, drugs, and bioactives and have identified 81 compounds, thus far, having positive effects on sperm motility. These hits are currently being evaluated and categorised in terms of their known, unknown or novel mode-of-action and medicinal chemistry tractability. Furthermore, screening of the National Phenotypic Screening Centre's 106,000 library of lead-like small molecules is ongoing and has already identified multiple different chemotype clusters. Compounds fulfilling our progression criteria will be progressed by testing the activity of analogues, structure-activity relationship determination, exploratory chemistry and mode-of-action studies. For chemotypes where no targets are known, proteomics-based target deconvolution techniques will be deployed, to help advance these hits.

P004 Synchronisation of sperm dynamics: modification in density gradient centrifugation improves normal motility indices of sperm

Alhilfi Haitham¹; Mohammed Amal²; Gomez-Martinez Judith³

¹Al-Qadisiyah University, Iraq; ²Al-Nahrain University, Iraq; ³Hospital Universitari i Politècnic La Fe, Valencia, Spain

Background: Sperm dynamics is affected by viscosity of surrounding medium and the beat frequency between adjacent sperm flagella (1). Density Gradient Centrifugation (DGC) is a technique used in the preparation of sperm for in vitro fertilisation.

Aim: To determine the effect of re-centrifugation of washed sperm pellets in density gradient colloids on synchronisation of sperm motility.

Methods: Frozen bovine semen samples (n=8) were divided into two equal aliquots (designated A and B) and separately thawed and centrifuged in 45/90% gradient colloid. The resultant sperm pellets were washed and centrifuged with fertilisation medium (TALP medium supplemented with caffeine 1.39mM). Then pellets of aliquot A were recentrifuged with the same gradient colloid and pellets of aliquot B recentrifuged only with medium. All pellets were then rewashed with medium, centrifuged again and subjected to evaluation. Chi-squared analysis was performed to test differences post-sperm preparation between conventional and modified DGC.

Outcomes: Re-centrifugation of sperm pellets in the density gradient colloid increased the percentage of total motile spermatozoa (75% vs 62.50%; P=0.003), progressively motile spermatozoa (54.63% vs 43.50%; P=0.001) and average sperm velocity $\mu\text{m/s}$ (26.75 vs 20.13; P<0.05) post preparation. No significant difference in total concentration of spermatozoa and live spermatozoa was observed.

Conclusion: Around 12% increase in progressively motile sperm was seen after re-centrifugation of sperm with the density gradient colloid. This may indicate that sperm cooperate with each other and synchronise their dynamics through the sliding friction of adjacent flagella in the colloidal solution that energizes low motility sperm. This important finding prompts further large-scale studies to investigate whether low-motile sperm acquire Ca^{+} from fertilization medium after initial DGC, and whether the sliding friction of flagella works as a trigger of sperm motility post Ca^{+} acquisition during the second DGC.

1- Yang Y, Elgeti J, & Gompper G. Cooperation of sperm in two dimensions: synchronization, attraction, and aggregation through hydrodynamic interactions. *Phys Rev E Stat Nonlin Soft Matter Phys.* 2008 Dec;78(6 Pt 1):061903. PMID: 19256864

P005 Does inducing capacitation change sperm metabolism?

Georgiou Merilin; Gruszyk Evie; Edney Samuel; Nevin Sean; Reynolds Steven; Pacey Allan

University of Sheffield

Background: Sperm undergo capacitation in order to achieve fertilisation, but how they supply energy during this process is not fully understood. Glycolysis and oxidative phosphorylation (OxPhos) in sperm can be measured using Magnetic Resonance Spectroscopy (MRS) by conversion of ^{13}C -glucose to ^{13}C -lactate or ^{13}C -bicarbonate respectively (1). The aim of this study was to identify whether capacitation changes sperm metabolism.

Methods: Twenty three ejaculates were washed using 40% Percoll/EBSS (Earle's balance salt solution) gradients. Washed sperm aliquots were mixed with the capacitation promoters Human Serum Albumin (HSA), Progesterone (P4) or HSA+P4 (control sample: EBSS) along with $^{13}\text{C}_6$ -glucose and antibiotics and incubated for 4 hours at 37°C and 5% CO_2 . ^{13}C -spectra were acquired using a 9.4T MRS spectrometer. Lactate and bicarbonate integrals were normalised to vital sperm concentration. Motility, vitality and capacitation status (CTC: chlortetracycline staining) were determined at time zero (T0) and 4h (T4).

Results: No significant changes in vitality or motility were observed between T0 and T4, although these were higher for both HSA containing samples than the control. CTC patterns were subjective and did not correlate with progressive motility, except for a negative slope for HSA+P4. ^{13}C -lactate was observed in all incubations, with significant positive correlations to vital sperm concentration. There were no significant differences in lactate integrals for the capacitation groups. Examining the spectra revealed that bicarbonate was more prevalent in HSA/HSA+P4 samples (both 7/15) compared to control (7/23) and P4 (3/16). Despite the increased prevalence in HSA containing samples normalised bicarbonate integrals were not significantly different.

Conclusions: Incubating sperm with HSA appears to cause an increased prevalence of OxPhos but does not influence glycolysis (lactate). Capacitation inducers did not change the percentage motility or capacitation. This may be effected by differing sperm phenotypes in the cohort and subjectivity in CTC observations. More reliable measures of capacitation are being investigated along with further sample incubations.

1. Calvert SJ, Reynolds S, Paley MN, Walters SJ, Pacey AA. Probing human sperm metabolism using ¹³C-magnetic resonance spectroscopy. *Mol Hum Reprod.* 2019;25(1):30-41.

P006 CASA and flow-cytometric parameters of semen for use in bovine in vitro embryo production (IVP)

Dodd Phillipa C¹; Burton Katie L²; Statham Jonathan ME²; Tutt Desmond AR¹; Sinclair Kevin D¹

¹School of Biosciences, University of Nottingham; ²RAFT Solutions Ltd

Background: Combined computer-assisted semen analysis (CASA) and flow-cytometry (FACS) has been advocated as a means of assessing fertility of semen in bovine artificial insemination [1,2,3]; it's efficacy for bovine in vitro fertilisation (IVF), however, is not known. CASA (morphological and motility assessments) and FACS (acrosome and mitochondrial physiology) was used to characterise semen from two sires (designated A and B) in IVP which assessed three concentrations (10, 50 and 100 µg/mL) of heparin during IVF.

Methods: CASA and FACS analyses of frozen-thawed bovine spermatozoa, both pre- and post-swim-up, was undertaken on three biological replicates according to [1]. IVF of abattoir derived oocytes followed in four-replicated experiments. Cleaved and non-cleaved zygotes were assessed on Day 2. Blastocyst development was assessed on Day 8. Proportions were analysed by logistic regression assuming binomial errors. Remaining data was analysed by ANOVA.

Results: For both sires, swim-up improved ($P < 0.01$) sperm parameters for motility, including straight-line velocity (VSL) and progressive motility, with less effect on sperm morphology and physiology. However, swim-up was found to reduce ($P < 0.001$) the percentage pyriform heads in Sire B (12.0 to 4.7%) but not Sire A (4.0 to 5.0%) (SED=0.62). Polyspermy was evident for Sire B only, and increased with heparin dose (0, 6.1 and 16.7%) during IVF. Cleaved of matured oocytes following IVF was greater ($P = 0.016$) for Sire A than Sire B (0.79 ± 0.027 vs 0.68 ± 0.030), but blastocysts of cleaved were greater ($P = 0.042$) for Sire B than Sire A (0.33 ± 0.028 vs 0.25 ± 0.024), such that mean proportion blastocysts of matured were similar for both sires and unaffected by heparin dose.

Conclusion: Tentatively, post swim-up VSL appears to offer the best marker of sperm fertilisation ability, but this requires confirmation with additional sires. Swim-up enriched the population of quality spermatozoa for both sires leading to similar levels of embryo development. Funder: BBSRC (BB/R007985/1).

1. Spilman, MW, Burton, KL and Statham, JME. Semenrate: the use of computer-assisted semen analysis and flow cytometry for objective bovine semen analysis in the United Kingdom. *Reprod. Fertil. Develop.* 2016; 29: 116-116.

2. Sellem E, Broekhuijsen ML, Chevrier L, Camugli S, Schmitt E et al. Use of combinations of in vitro quality assessments to predict fertility of bovine semen. *Theriogenology* 2015; 84:1447-1454.

3. Gliozzi TM, Turri F, Manes S, Cassinelli C and Pizzi F. The combination of kinetic and flow cytometric semen parameters as a tool to predict fertility in cryopreserved bull semen. *Animal* 2017; 11:1975-1982.

P007 Human sperm [Ca²⁺]_i oscillations require regulation of CatSper by Vm

Publicover Stephen¹; Torrezan Elis¹; Brown Sean²

¹University of Birmingham; ²University of Abertay

Background: CatSper, an ion channel unique to the sperm flagellum, is activated by voltage, elevated pHi and (in human sperm), many small organic molecules, including progesterone (P4). In the presence of P4 a subset of sperm display large [Ca²⁺]_i oscillations that regulate flagellar behaviour (1) and inhibit acrosome reaction (2). Though pharmacological manipulations indicate involvement of stored Ca²⁺ in these oscillations, their persistence requires extracellular Ca²⁺, suggesting a role for Ca²⁺ influx (1,2). We have used Ca²⁺-imaging to investigate the role of CatSper in generation of [Ca²⁺]_i oscillations in human sperm.

Method: Capacitated human sperm, loaded with fluo-4, were immobilised on poly-D-lysine-coated coverslips. Cells were observed using time-lapse fluorescence microscopy (0.1-2.5 Hz; LED illumination; EMCCD camera) whilst continuously superfused with sEBSS (25°C). To manipulate/clamp membrane potential (V_m), we used the K^+ -ionophore valinomycin (1 μ M). Direct assessment of V_m (whole-cell current-clamp) showed that valinomycin held V_m at -60 mV and +2.5 mV in standard and high (100 mM)- K^+ sEBSS respectively.

Results: $[Ca^{2+}]_i$ oscillations originated in the flagellum, spreading to the neck and head (latency of 3-4 s) where amplitude was significantly increased ($P < 0.01$). To investigate involvement of CatSper, we used the semi-specific inhibitor RU1968-F1 (3). 10 and 30 μ M RU1968-F1 after P4 caused immediate arrest of activity in 30% and 92% of oscillating cells respectively. Pretreatment of sperm with valinomycin had no effect on P4-induced Ca^{2+} influx ($P = 0.8$; 8 experiments) but application of valinomycin to P4-pretreated sperm suppressed activity in 82% of oscillating cells ($n = 257$) and significantly reduced both amplitude and frequency of persisting oscillations ($p < 0.01$). Oscillations re-started upon valinomycin washout. Application of valinomycin in high- K^+ sEBSS inhibited oscillations in only 25% of cells ($n = 114$; $P < 0.0001$).

Conclusion: Oscillations originate in the flagellum by voltage-regulated activity of CatSper, but are amplified in the sperm head/neck, probably due to Ca^{2+} -store mobilisation.

1. Harper CV, Barratt CL, Publicover SJ. Stimulation of human spermatozoa with progesterone gradients to simulate approach to the oocyte. Induction of $[Ca^{2+}]_i$ oscillations and cyclical transitions in flagellar beating. *J Biol Chem.* 2004; 279:46315-25. 2. Mata-Martinez E, Darszon A, Trevino CL. pH-dependent Ca^{2+} oscillations prevent untimely acrosome reaction in human sperm. *Biochem Biophys Res Commun.* 2018; 497:146-52. 3. Rennhack A, Schiffer C, Brenker C, Fridman D, Nitao ET, Cheng YM, et al. A novel cross-species inhibitor to study the function of CatSper Ca^{2+} channels in sperm. *Br J Pharmacol.* 2018;175:3144-61.

P008 Rare pathogenic mutations identified by whole exome sequencing of 26 male patients who experienced IVF failure

Kane Shruti¹; Lester Douglas¹; Barratt Christopher²; Martins da Silva Sarah²; Brown Sean¹

¹Abertay University; ²University of Dundee

Background: Unexplained infertility affects 15% of couples however genetic analysis offers diagnostic potential. We performed whole exome sequencing of 26 sub-fertile patients to identify underlying genetic defects in patients.

Methods: DNA extracted from the blood of 26 unrelated, sub-fertile men of known fertility outcomes were sequenced using the Illumina version 5 whole exome sequencing kit. Raw fastq files were subjected to quality filtration and aligned to reference human genome (hg38). Allele specific variants were picked after duplicate removal and base recalibration using the GATK HaplotypeCaller. Variants were filtered using a training dataset and the variant quality scores were recalibrated. Rare variants from the exonic region were identified, and pathogenicity of the mutations were predicted using pathogenicity prediction algorithms. Results: Two different novel frame-shift insertion, homozygous mutations were seen in the coiled-coil domain protein 8 (CCDC8) in 7 unrelated patients, who showed complete failed fertilization rates after IVF treatment. In addition a previously reported homozygous 2bp deletion, causing a frame-shift mutation in the β -defensin 126 (DEFB126) gene, was also identified in 6 patients. Sanger sequencing was carried out to confirm the presence of these mutations in patient samples.

Conclusions: The CCDC proteins contain a conserved coiled-coil motif that is responsible for protein folding. Homozygous loss of function of other genes from the CCDC family have been shown to cause morphological abnormalities in the sperm tail. Future experiments on the CCDC8 knockout sperm should detect morphological abnormalities. For example the use of transmission electron microscopy (TEM), will detect any abnormalities in the formation of the dynein arms in sperm flagella. The detected DEFB126 mutation, is a common variant, that has previously been implicated in the efficient movement, of sperm, in the female reproductive tract. Sperm penetration assays, will therefore need to be performed, on affected patients, to provide evidence for functional failure.

P009 Antigen unmasking of phospholipase C zeta in human sperm does not alter potential diagnostic outcome in patients undergoing fertility treatment

Kashir Junaid¹; Mistry Bhavesh²; BuSaleh Lujain³; AbuDawas Reema³; Nomikos Michail⁴; Ajlan Ahmed²; Abu-Dawud Raed²; AlYacoub Nadya²; AlHassan Saad²; Lai F. Anthony⁴; Assiri Abdullah²; Coskun Serdar²

Background: Mammalian oocyte activation is mediated via a series of intracellular calcium (Ca²⁺) oscillations induced by sperm-specific phospholipase C zeta (PLCζ). However, significant concern surrounds PLCζ antibody specificity and detection protocols. Antigen unmasking/retrieval protocols (AUM) improved the visualization efficacy of PLCζ in mammalian sperm. However, this enhancement seemed patient specific in human sperm, raising concerns that AUM may alter diagnostic outcome. Herein, we examined whether application of AUM affected observed PLCζ fluorescence levels or localisation patterns in relation to routinely examined parameters of sperm health.

Methods: A total of 65 patients were recruited following informed written consent. Sperm concentration, motility, morphology, and semen volume were recorded and samples subject to density gradient washing. Sperm were subject to immunofluorescence and immunoblotting using two distinct antibodies with a high degree of specificity against PLCζ. Excess sperm not required for treatment was used, and subject to immunofluorescence with and without AUM using two distinct antibodies with a high degree of specificity against PLCζ.

Results: AUM significantly increased the proportion of sperm exhibiting PLCζ fluorescence regardless of specific pattern, while significantly decreasing the proportion of sperm not exhibiting any PLCζ. As previously observed, AUM significantly increased fluorescence observed compared to without AUM, while two-way ANOVA indicated that patient type determined increase or decrease of quantified fluorescence levels. However, proportions of localisation patterns recorded with or without the use of AUM did not differ in any optimal semen parameter examined, nor did AUM alter levels of variance observed. Correlations between PLCζ fluorescence and parameters of sperm health also remained unaffected by the application of AUM.

Conclusions: Collectively, such results indicate that AUM methodology increased the visual efficacy of PLCζ in human sperm, without altering the potential diagnostic outcome, thus representing a valuable methodological tool.

P010 The role of vasopressin in human sperm function

Tomova Ana-Maria¹; Hughes Jessica²; Hunter Helen²; Brison Daniel²; Ashworth Jason¹; Murgatroyd Chris¹; Carroll Michael¹

¹Department of Life Sciences, Faculty of Science and Engineering, Manchester Metropolitan University; ²Department of Reproductive Medicine, Manchester University NHS Foundation Trust

Background: Neuropeptides play an essential role in reproduction, with many of their receptors found throughout both male and female reproductive tracts (1). Vasopressin (VP), with a key role in homeostasis, is also important in regulating reproductive behaviours (2). Though two relatively old studies have detected VP in human and Bull semen little is known about its possible role in human sperm function (1, 3, 4). The present study aims to explore the role of VP on human sperm function.

Methods: Semen samples were obtained from consenting males following NHS and Faculty approval. Vasopressin receptor 2 (VPR2) expression on sperm was analysed via immunostaining and seminal plasma vasopressin concentrations detected by ELISA. Sperm motility and kinematics were measured via CASA (SAMI). Sperm calcium measurements were carried out using fluorescent microplate assays. Acrosome-reacted sperm were detected using SEM. Aquaporin-2 (AQP2) expression in sperm was detected using dot blots and western blots.

Results: We confirm the presence of VP in human semen; at concentrations of 143.4 -- 2827.9 pg/ml (n = 74). VPR2 was found localised to the sperm acrosomal region. Treatment of human sperm with desmopressin and VP significantly modulated motility, linearity and curvilinear velocity (p = < 0.05) and induced a significant calcium response (p = < 0.05). VP-treatment led to a 3-fold increase in acrosome-reacted sperm (Vehicle 1.8%, VP 6.2%). Both glycosylated and non-glycosylated isoforms of AQP2 were found in untreated, vasopressin treated and capacitated sperm.

Conclusions: The variations in semen VP concentrations, together with sperm responses to VP and agonist treatments and the occurrence of VPR2 and AQP2 in human sperm suggests a possible mechanistic role of VP in sperm function. Further work is needed to understand the role of VP in fertility and the potential use of VP as a biomarker of reproductive health.

1. Uhlen M, Fagerberg L, Hallström BM, Lindskog C, Oksvold P, Mardinoglu A, et al. Tissue-based map of the human proteome. Science. 2015;347(6220):1260419.

2. Knafo A, Israel S, Darvasi A, Bachner-Melman R, Uzefovsky F, Cohen L, et al. Individual differences in allocation of funds in the dictator game associated with length of the arginine vasopressin 1a receptor RS3 promoter region and correlation between RS3 length and hippocampal mRNA. *Genes Brain Behav.* 2008 Apr 1;7(3):266–75.
3. Brotherton J. Vasopressin: another pregnancy protein in human seminal plasma: Vasopressin: Ein weiteres Schwangerschaftsprotein im menschlichen Seminalplasma. *Andrologia.* 1990;22(4):305–7.
4. Lundin S, Toresson G. Immunoreactive vasopressin in bull seminal fluid. *Horm Metab Res.* 1987;19(12):629–31.

P011 Exploring sperm DNA damage in response to phosphodiesterase inhibitor stimulation of motility

Sharpe Abigail; Miller David

University of Leeds

Background: There are concerns that IntraCytoplasmic Sperm Injection (ICSI) is being used unnecessarily to overcome poor sperm motility with potential long-term consequences(1). Phosphodiesterase inhibitors, such as pentoxifylline (PF) are promising compounds that could be used to improve sperm motility and fertilisation rates without the need for ICSI; however little information on their impact on sperm DNA integrity is available. Methods The effects of increasing doses of PF on sperm motility, DNA integrity and viability were examined on samples of frozen bovine sperm with the aid of a computer assisted semen assessment platform (CASA; SCA-Microm) using, respectively, the motility and sperm DNA dispersion (HALO) assay modules alongside eosin-nigrosin staining(2). Effects of PF were compared to the oxidative effect seen with increasing doses of hydrogen peroxide, where a small HALO area suggests increasing DNA fragmentation. All data were analysed with GraphPad Prism software. Results Overall motility increased with higher doses of PF (0-20mM), with a peak effect of 49.7% motility at 10mM PF compared to 38.1% at 0 PF. Rapid motility rose to 29.8% at 10mM PF compared to 11.2% at 0 PF and hyperactive motility, defined as both the amplitude of lateral head displacement and curvilinear velocity were significantly increased ($p < 0.01$). No detrimental effects on either viability (88.4% vs 92.4% at 0 and 20mM PF respectively, $p = 0.29$) or the levels of DNA fragmentation were seen, where mean HALO areas were similar on exposure to 0, 10mM and 20mM of PF (134.6, 135.7 and 137.0 μm^2 respectively; $p > 0.05$). This was in contrast to hydrogen peroxide exposure (0 to 750 μM), which led to a decrease in overall motility (37.7% to 24.6% respectively) and viability (85.6% to 67.1% respectively), as well as raising the level of DNA fragmentation (169.40 μm^2 to 133.5 μm^2 respectively, $p < 0.01$). Conclusions Increasing doses of PF up to 10mM improved sperm motility, and hyperactivity without any detrimental effects on viability and DNA integrity. As hydrogen peroxide had deleterious effects on all three measures, we can conclude that PF may improve sperm motility without fear of compromising sperm DNA damage, potentially avoiding the use of ICSI in selected patients.

1. Esteves SC, Roque M, Bedoschi G, Haahr T, Humaidan P. Intracytoplasmic sperm injection for male infertility and consequences for offspring. *Nature Reviews Urology.* 2018 01 Sep;15(9):535-62.
2. Fernandez JL, Muriel L RM, Goyanes V, Vazquez R, Alvarez JG, . The sperm chromatin dispersion test: a simple method for the determination of sperm DNA fragmentation. *Journal of Andrology.* 2003;24(1):59-66.

P012 Effects of freeze-thawing cycles and storage length on testes and sperm morphology

Mitchell Rosie; Berneau Stephane; Brassey Charlotte

Manchester Metropolitan University

Background: Zoos and museums are valuable sources of animal cadaveric material, including genital tissues, to address important questions within the fields of animal husbandry and evolutionary biology. Yet such specimens are often subjected to suboptimal long-term storage and freeze-thaw cycles. In this study, we aim to evaluate the effects of storage length and freeze-thaw cycles on testes and sperm morphology.

Method: 17 Wistar rats within 2 age cohorts (8-9 and 39-40 weeks) were dissected and both testes and epididymides were collected. One testis per pairs was 10%NBF-fixed after a 'simulated damage' protocol of 2 freezing-thawing cycles within a 2-week ($n = 6$), a 4-week ($n = 6$) or a 6-months ($n = 4$) freezing length. The control paired testis was immediately

fixed after dissection. All testes were paraffin-embedded and sectioned. Slides were haematoxylin-eosin stained and imaged to evaluate the testis structure. Sperm was retrieved by mincing fresh and frozen epididymides and fixed with 4%PFA onto 10mm² silicon wafers (n=5) and imaged using a scanning electron microscope. All image analyses were performed using ImageJ.

Results: Our initial results suggest the alteration in testes structure in frozen samples. All 'simulated damage' conditions show a similar damage pattern of increased interstitial space and inconsistency in seminiferous tubules structure, suggesting the absence of storage length effect. Additionally, a 43% increase in the percentage of sperm heads detached from the tail in the frozen sperm samples compared to the control. However, the remaining intact sperm showed no morphological differences between fresh and frozen conditions.

Conclusion: Our results suggest that specimens stored at -20°C should be used with caution for morphology analysis. Freeze-thaw cycles generated irreversible damages to the internal structure of the testis. However, extracted sperm maintain their gross cell morphology despite freeze-thaw damage, highlighting the importance of natural history collections to the study of comparative animal reproduction.

P013 The effect of lifestyle on human sperm count and progressive motility: a longitudinal study

Berneau Stéphane¹; Noori Muaamar²; Shehzad Mahaah¹; Tomova Ana-Maria¹; Al-Gazi Maha K.A.¹; Brison Daniel R.³; Povey Andrew²; Carroll Michael¹

¹Department of Life Sciences, Faculty of Science and Engineering, Manchester Metropolitan University; ²Centre for Epidemiology Division of Population Health, Faculty of Biology, Medicine and Health, University of Manchester; ³Maternal and Fetal Health Research Centre, Faculty of Biology, Medicine and Health, University of Manchester

Background: An overall decline in total sperm count over the last forty years was recently reported in Western countries¹. Moreover, sperm parameters are highly variable with time in repeated samples. Causes of this decline and individual variations are still unknown and lifestyle factors including diet may be contributing factors. In the present study, we aim to investigate the variation of human sperm parameters in a general public cohort over time and to establish the effect of lifestyle and diet on this variation.

Methods: 20 male donors from Manchester, UK were enrolled in a 6-month longitudinal study. 15 participants completed the study, provided a semen sample and completed a lifestyle/dietary questionnaire at each visit (fortnightly over 6 months). Semen parameters such as total sperm count and progressive motility (CASA) were assessed in samples within an hour after ejaculation (n=180). Correlations between lifestyle and sperm parameters were assessed using linear models adjusted for age, body mass index and abstinence.

Results: Our data demonstrate that total sperm count and progressive motility were respectively by 48.5% and 20.1% variable in our cohort. Sporadic variations in lifestyle and dietary parameters were observed in overall individual routines. Total sperm count was significant positively correlated with moderate exercise (r=0.15) and high-fat dairy (r=0.19), cruciferous vegetables (r=0.17) and pulses (r=0.15) consumption. Sperm progressive motility was significant positively correlated with vegetable oil (r=0.32), spicy products (r=0.17) and caffeinated sugar beverages (r=0.26) consumption. Both sperm parameters were significant negatively linked to poultry consumption (r=-0.31).

Conclusion: In this study, only the moderate exercise as a lifestyle factor had a low impact on sperm count. Moreover, food categories which weakly influenced total sperm count tended a plant-based diet with reduced poultry consumption. Identification of these influences on sperm parameters enables us to further investigate the effect of dietary patterns' choice on male fertility.

1-Levine H., Jørgensen N., Martino-Andrade A., Mendiola J., Weksler-Derri D., Mindlis I., Pinotti R. & Swan S.H. 2017. Temporal trends in sperm count: a systematic review and meta-regression analysis, Human Reproduction Update, Volume 23, Issue 6, Pages 646-659

P014 Case report: The first infant born following treatment of a HIV positive patient at the Herts and Essex Fertility Centre

Syed Najma¹; Naik Rahul¹; Fryer Hayley; Richardson Lucy

¹Herts and Essex Fertility Centre

Background: We report the first human immunodeficiency virus (HIV) sperm washing case in our clinic which resulted in the birth of a healthy female in August 2019. A 34-year-old male patient had been diagnosed with HIV in 2010 and was on highly active antiretroviral therapy (Isentress and Truvada). The female was HIV negative. The couple had been referred as they had been trying to conceive for 18 months without success.

Method: A semen sample was prepared and assessed against WHO 1999 criteria. A density gradient was performed and the sample was centrifuged at 1500 rpm for 15 minutes to separate the HIV-infected seminal fluid from the sperm. The sample subsequently underwent three sperm washes at 1500 rpm for 10 minutes each. Following the final wash, a small aliquot of the residual sperm pellet was removed and sent for virology testing to assess for the presence of HIV. After it was determined that HIV was not present in the prepared sample, it was cryopreserved for use in treatment at a later date. The semen sample was thawed and Intra-Cytoplasmic Sperm Injection (ICSI) was performed on 21 mature oocytes following egg collection.

Results: A fertilisation rate of 76% was achieved. A single embryo (3BB) was transferred on day 5 with 2 blastocysts cryopreserved on day 5. The patient delivered a healthy female infant following spontaneous vaginal delivery at 39 weeks with no complications.

Conclusion: The high fertilisation rate and good embryo quality indicates that additional sperm washing does not have a negative effect on sperm. Consequently, repetitive sperm washing can be used to achieve pregnancy in HIV-infected patients whilst reducing the risk of HIV transmission.

P015 Clinical outcomes following deferred embryo transfer in stimulated IVF/ICSI cycles: a retrospective study

Dickinson Bonnie¹; Carroll Michael²; Wilson Paul¹; Gibbons Corrina¹

¹Bristol Centre for Reproductive Medicine; ²Manchester Metropolitan University

Background: Studies have indicated controlled ovarian stimulation (COS) in assisted reproductive techniques (ART) may have a negative effect on the endometrium and endometrium-embryo receptivity, thus reducing implantation rates of embryos transferred in a fresh cycle. Some studies have showing that deferring embryo transfer (ET) by freezing all suitable embryos for subsequent use in a frozen embryo transfer (FET) may result in better outcomes. It is thought that this allows the uterine environment to recover from the effects of COS. Advances with vitrification of embryos has allowed for increased implementation of deferred ET.

Aim: To investigate the clinical outcomes of ART when comparing deferred ET with fresh ET.

Methods: A retrospective study of ART patients aged under 38 (n=2482) who undertook In vitro fertilization (IVF) or Intracytoplasmic sperm injection (ICSI) treatment over a 4-year period who underwent COS, oocyte retrieval, fertilization and embryo culture. Upon selection of suitable embryo development, patients had either fresh ET (n=2316) or vitrification (n=166) on day 3 or day 5.

Results: A significant increase in clinical pregnancy rates (CPR) was noted for IVF patients who undertook a deferred ET when compared to fresh ET for their 1st (74.2% vs 46.5%, p=0.05).

Conclusion: These results suggest that a deferred ET approach may result in better higher CPRs for patients aged <38 years undergoing IVF treatment. Further investigation through large, randomised controlled trials should be undertaken before this is implemented in routine clinical practice.

P016 Do supplementary intake of vitamins and antioxidants have a role in improving male fertility outcomes?

Balakumar Vidhya; Martins Da Silva Sarah

NHS Tayside

Objective: One in seven couple worldwide have difficulties in conception and male factor accounts for almost 50% of this. Oxidative stress predominantly caused by life exposures is seen as an important contributing factor. The recent Cochrane review (Smits et al 2019) suggests that antioxidants may be a valid intervention for male infertility, however, commercial formulations are largely untested (Martins da Silva RBM online 2019). The aim of this study was to assess life exposures, dietary supplements and sperm parameters among a representative population of male patients attending tertiary fertility services.

Methods: A questionnaire was completed by male patients attending our Assisted Conception Unit between June 2019 to August 2019. Data collection included age, postcode, BMI, smoking, alcohol consumption, and intake of dietary oral supplements as well as semen parameters. Results: 120 men completed the questionnaire. The responses were divided into 5 groups using Scottish Index of Multiple Deprivation (SMID) quintile scores, with each group evenly represented. Overall, 46 (38.3%) were taking oral vitamin and dietary supplements (VDS), although only 56% of these were taking VDS specifically for fertility purposes. VDS intake was high in those with lower SMID (most deprived) groups, and highest in group 2 (57%). Men who consumed at least 5 home cooked meals were predominantly from groups 4 & 5. Almost all men were non-smokers, as per Scottish NHS funding access criteria, and there was no statistical difference in the BMI between the groups.

Conclusion: Our results indicate that a large proportion of men undertaking assisted conception may be consuming VDS, but that only half do so primarily for fertility reasons. Men from more deprived backgrounds were more likely to take VDS and less likely to eat home cooked meals, which is an important healthcare message. Further large randomised placebo-controlled trials are required to understand the benefit of VDS.

P017 A comparison of microscopic semen assessment as performed by a manual operator and the LensHooke™ X1 Pro

Fryer Hayley; Hejazi Angela; Naik Rahul; Richardson Lucy

Herts and Essex Fertility Centre

Background and objectives: Manually performed semen analyses are often criticised due to the potential for human error and the presence of inter-operational variance, especially when morphology is considered. The LensHooke™ X1 Pro is a computer-aided semen analysis (CASA) device which aims to eliminate subjectivity during semen assessment. This study's objective was to investigate the performance of the LensHooke™ X1 Pro when evaluating microscopic semen parameters.

Methods: A prospective repeated measures study compared assessment of semen concentration (m/ml), motility (% motile) and morphology (% normal forms (NFs)) between a manual operator and the LensHooke™ X1 Pro using WHO 2010 criteria. The manual operator followed laboratory SOPs whilst the LensHooke™ X1 Pro was used in accordance with manufacturers guidelines. A paired samples T-Test was used to determine statistical differences and significance was accepted at $P < 0.05$.

Results: No significant difference was found between the manual operator and LensHooke™ X1 Pro assessment of concentration ($P=0.29$) or motility ($P=0.23$). A highly significant difference was found in assessment of morphology ($P=0.001$), with the CASA device consistently reporting lower percentage NFs than the manual operator. Overall, the LensHooke™ X1 Pro and manual operator differed in assessment of normalcy in 44% of patients in a way which would alter clinical treatment decisions i.e. whether IVF or ICSI would be recommended.

Conclusion: The LensHooke™ X1 Pro assessed concentration and motility similarly to the manual operator and could therefore be confidently used for these purposes. It is unclear whether the significantly different morphological evaluations reflect a fault in the CASA device or demonstrate the human error which the LensHooke™ X1 Pro aims to eliminate. A study comparing clinical outcomes when treatment decisions are based either on the semen assessment of a manual operator or LensHooke™ X1 Pro would help determine which of these possibilities is more likely.

LensHooke™ X1 Pro "bonraybio, 4F., No.118, Gongye 9th Rd., Dali Dist., Taichung City 41280, Taiwan

P018 Uncertainty and variation in sperm morphology preparation and assessment across UK laboratories

Allan Andrew¹; Tomlinson Mathew²; Gregoire Rachel¹

¹Hewitt Fertility Centre; ²Nottingham University Hospital

Introduction: Sperm morphology is considered to be a powerful indicator of male fertility potential. However, recent powerful studies in IVF and ICSI have failed to replicate work from the 1980s and suggest that morphology assessed according to 'strict criteria' has no clinical value (1).

Method: A questionnaire was sent to members of ABA (Association of Biomedical Andrologists) and UK NEQAS (National External Quality Assessment) participants to establish method-variation in morphology assessment across UK laboratories. Respondents were also asked to assess 20 stained-sperm images using current WHO recommended methods

Results: Of the 80 responses, 26 (32.5%) don't follow WHO recommendation of using fixed stained slides (PAP, Shorr, Dif Quik) for morphological assessment. There were clear differences in how long laboratories leave slides to air dry with only 13 (16.2%) labs immediately fixing slides once they are dry, 6 (9.8%) leaving slides overnight and 34 (42%) labs having no standardised fixation time. Only 3 labs (3.8%) measured sperm dimensions using an eyepiece graticule, yet 59 (77%) classify borderline forms according to WHO. From the 20 sperm images, 15 resulted in significant uncertainty amongst users with no agreement in whether a sperm was normal or abnormal, or in type of abnormality reported.

Conclusion: There is clearly a lack of standardisation in the assessment of sperm morphology across the UK. However, the 75% disagreement from a series of stained images suggests that methodology is only a contributory factor to any overall level of uncertainty and that the industry as a whole has a problem in recognising true sperm defects from artefacts. It is perhaps therefore no surprise that large prospective trials in IVF patients determine the parameter to be of little clinical value.

1. van den Hoven L, Hendriks J, Verbeet J, Westphal J, Wetzels A. Status of sperm morphology assessment: an evaluation of methodology and clinical value. *Fertility and Sterility*. 2015;103(1):53-58.

P019 Impact of chemerin on sperm function and embryo development in broiler breeder chicken

Estienne Anthony; Bourdon Guillaume; Grandhaye Jérémy; Caldas-Silveiras Erika; Ramé Christelle; Barbe Alix; Chahnamian Marine; Ganier Patrice; Froment Pascal; Dupont Joelle

INRA

Chemerin is an adipokine that plays a role in several biological processes such as adipogenesis and metabolism. Recent findings showed a negative correlation between plasma chemerin and female fertility parameters including egg hatchability. So, we investigated the effects of chicken recombinant chemerin (ReCChem) on rooster sperm since the fertility of these animals presents a agronomic interest. By immunoblot and qPCR, we have shown that chemerin and its receptors CMKLR1, CCRL2 and GPR1 were expressed within the testis from the embryo stages to the adulthood, with a lowest expression in adult testis. By using chicken specific ELISA and immunoblot, we observed that chemerin levels were higher in blood than in seminal plasma. A qualitative analysis of roosters sperm highlighted a negative correlation between seminal plasma chemerin levels and the percentage of motility, progressive motility and the spermatozoa concentration. Furthermore, the percentage of static spermatozoa was correlated with chemerin concentration in seminal plasma. After in vitro experiments of rooster sperm treated with ReCChem and/or with an anti-CMKLR1 antibody and measurement of the massal motility of sperm, we showed that ReCChem inhibited sperm massal motility and this effect was abolished when sperm was pre-incubated with the anti-CMKLR1 antibody. After In vivo experiments of fresh rooster sperm incubated with ReCChem and used for artificial insemination (AI), we observed a negative effect of chemerin on eggs fertility for the three first days after AI. Then eggs' fertility became identical between the tested conditions, suggesting a transitory negative effect of chemerin on sperm. Taken together, seminal chemerin levels are negatively associated to the rooster fertility and chemerin produced locally by testis or male tract could negatively affect sperm quality through CMKLR1. Thus, the chemerin system is a negative regulator in male reproductive function in chicken. This project is supported by the Région Centre "PREVADI".

P020 Identification of miR-202 in bull testis using in situ hybridisation

Mohammed Bushra T¹; Donadeu Xavier²

¹College of Veterinary Medicine, University of Duhok, Iraq; ²The Roslin Institute and R(D)SVS, University of Edinburgh, Scotland, UK

The availability of bulls with optimum spermatogenic capacity is pivotal to counteract the decline of fertility in modern cattle systems and to produce healthy offspring. Successful development and maturation of male gametes relies on complex hormonal, cellular and molecular mechanisms. Studies have demonstrated that small non-coding RNAs including miRNAs are critically involved in regulating the development and function of the male reproductive system (1). In our previous studies, we have assessed the functional roles of a subset of miRNAs including miR-202 in promoting ovarian cell growth and steroidogenesis in bovine (2). miR-202 has been identified in different species as a gonad specific miRNA involved in early gonad differentiation in the embryo (3). To further understand its involvement in testicular function in bovine, this study was designed to investigate the localisation of miR-202 in bull testicular cells. Bull testes were collected at an abattoir. miR-202 expression was quantified in paraffin embedded tissue samples using in situ hybridisation (miRCURY LNA microRNA ISH technology). We found that miR-202 was predominantly localised to Sertoli cells and all type of germ cells including spermatogonia and spermatocytes, suggesting that this miRNA may play a beneficial role in mediating testis development and may perhaps provide a good strategy to evaluate sperm for assisted reproduction in bovine.

1. Reza AMMT, Choi YJ, Han SG, Song H, Park C, Hong K, et al. Roles of microRNAs in mammalian reproduction: from the commitment of germ cells to peri-implantation embryos. *Biol Rev.* 2019; 2. Sontakke SD, Mohammed BT, McNeilly AS, Donadeu FX. Characterization of microRNAs differentially expressed during bovine follicle development. *Reproduction.* 2014;148(3). 3. Bannister SC, Tizard MLV, Doran TJ, Sinclair AH, Smith CA. Sexually Dimorphic MicroRNA Expression During Chicken Embryonic Gonadal Development1. *Biol Reprod.* 2009;

P021 Radio frequency electromagnetic radiation from cell phone causes defective testicular function in male wistar rats

Oyewopo Adeoye

University of Ilorin, Nigeria

Background: Cell phones have become an integral part of everyday life. As cell phone usage has become more widespread, concerns have increased regarding the harmful effects of radiofrequency electromagnetic radiation (RF-EMR) from these devices. The current study was undertaken to investigate the effects of the emitted radiation by cell phones on testicular histomorphometry and biochemical analyses.

Design and Method: Adult male Wistar rats weighing 180g-200g were randomly allotted to control; group A (switched off mode exposure), group B (1 hour exposure), group C (2 hours exposure and group D (3 hours exposure). The Animals were exposed to radiofrequency electromagnetic radiation of cell phone for a period of 28 days. Histomorphometry, biochemical and histological investigations were carried out.

Results: The histomorphometric parameters showed no significant change ($p>0.05$) in the levels of germinal epithelial diameter in all the experimental groups compared to the control group. There was no significant change ($p>0.05$) in cross section diameter of all the experimental groups compared to the control group. Group D rats showed a significant decrease ($p<0.05$) in lumen diameter compared to group B rats. There was an uneven distribution of germinal epithelial cells in groups B, C and D. However, there was degeneration of the epithelia cells in group D when compared to the control and group B rats. Sera levels of malondialdehyde (MDA) and superoxide dismutase (SOD), which are markers of reactive oxygen species (ROS) significant increase (MDA) and decrease (SOD) respectively in all the experimental groups compared to control group. Also sera levels of gonadotropic hormones (FSH, LH and testosterone) significantly decreased ($p>0.05$) in groups C and D compared to the control group.

Conclusion: The study demonstrates that chronic exposure to radiofrequency electromagnetic radiation of cell phone leads to defective testicular function that is associated with increased oxidative stress and decreased gonadotropic

References 1.Kesari KK, Kumar S, Behari J (2011) Effect of radiofrequencyelectromagnetic wave exposure from cellular phones on thereproductivepattern in male Wistar rats. Appl Biochem Biotechnol164: 546-559. 2.Çetkin M, Kızılkın N, Demirel C, Bozdağ Z, Erkiş S, et al. (2017)Quantitative changes in testicular structure and function in ratexposed to mobile phone radiation. Andrologia. 3.Saikhun J, Kitiyanant Y, Vanadurongwan V, Pavasuthipaisit K (1998)Effects of sauna on sperm movement characteristics of normalmen measured by computer-assisted sperm analysis. Int J Androl21: 358-363. 4.Kesari KK, Kumar S, Behari J (2010) Mobile phone usage and maleinfertility in Wistar rats. Indian J Exp Biol 48: 987-992. 5.Alaa JH, Aspinder S, Ashok A (2011) Cell phones and their impacton male fertility: fact or fiction.Fertil Steril 90: 125. 6.Roelands R (2010) Cellular phones and the skin. Dermatology207: 3-5. 7.Depinder F, Makkler K, Agarwal A (2007) Cell phones and maleinfertility:dissecting the relationship.Reprod Biomed Online 3:266-270. 8.Cheesbrough M (2006) District Laboratory Practice in tropicalcountries. Cambridge University press 131-132. 9.Agarwal A, Desai NR, Makker K, Varghese A, Mouradi R, et al.(2009) Effects of radiofrequency electromagnetic waves fromcellular phones on human ejaculated semen: an in vitro pilotstudy. FertilSteril 92: 1318-1325. 10.Kao SH, Chao HT, Chen HW, Hwang TI, Liao TL, et al. (2007)Increase of oxidative stress in human sperm with lower motility.Fertil Steril online publication ahead of print.

P022 Comparing sperm parameters in first and second IVF samples: Are there any improvements or is ICSI the last resort?

Ali Leilan; Besi Despoina; Rattos Annabel; Jansa Perez Marta; Salim Rehan

Wolfson Fertility Centre

The aim of this study was to determine whether it is beneficial for IVF patients, whose first semen sample exhibited abnormal parameters, to produce a second sample or to immediately convert to ICSI. In this retrospective study, 151 fresh IVF cycles (August 2013 - August 2019) were evaluated (mean male age: 37.4; range 26-54) where a second semen sample was requested due to first samples being below WHO reference values. The average volume, concentration and motility of first and second samples were calculated and compared. The number of IVF to ICSI conversions was noted and fertilisation rates (FRs) of IVF and ICSI cycles were compared. A paired t test and Fisher's Chi-square exact test were used whereby $P < 0.05$ was considered to be statistically significant. The second samples were of significantly smaller volume compared to the first samples (1.8ml vs 2.8ml, $p < 0.05$) and revealed pre and post-preparation analysis improvements in sperm concentration (45.6m/ml vs 43.5m/ml; 6.5m/ml vs 4m/ml), progressive motility (44.1% vs 41.5%; 82.2% vs 73.3%) and non-progressive motility (8.36% vs 7.7%; 2.16% vs 3.1%), but the differences were not statistically significant. Of the 151 cycles, 79 cycles were converted to ICSI due to low sperm quality in the second sample. FRs were comparable between IVF and ICSI cycles (72.8% vs 75.8%, $p > 0.05$). This study identifies significant improvements in sperm concentration and motility in second samples compared to the first on the day of egg collection. The data reassuringly shows that FRs were very similar for cycles that remained IVF compared to cycles converted to ICSI. This indicates low parameters in a first sample on the day of treatment are not an immediate indicator to convert to ICSI.

P023 Evaluation of early vasectomy failure rate between referral centres following the introduction of 2016 PVSA guidelines

Adamson Amber

Liverpool Women's Hospital

Background: Post vasectomy semen analysis (PVSA) determines if sperms are present in the semen following vasectomy. Although the accepted vasectomy failure rate is low (NHS recorded figure of 1%), it is possible. Early vasectomy failure can be attributed to surgical error or reduced interval between surgery and analysis. The 2002 BAS guidelines were superseded by the 2016 Post Vasectomy Guidelines for semen analysis and introduced clinically in 2017. The aim was to determine if an increased rate of early failed vasectomy observed at the centre is linked to surgical technique by referring surgeons or to a reduced interval to analysis post-vasectomy.

Methods: A retrospective analysis (January 2017-December 2018) of post vasectomy semen parameters. Vasectomy failure was defined as motile sperm observed in the primary sample. The study compared failed vasectomies from 4 referral groups (A,B,C,D) with a total of 1958 PVSA samples.

Results: There was significant difference when comparing all referral groups ($p < 0.022196$). Vasectomy failure was significantly higher in one referral group (Group A) when compared with all other referral groups ($p < 0.000644$). 53 patients had a PVSA between January-March 2017, performed 16 weeks following vasectomy. 1905 patients had PVSA

between April 2017 and December 2018, with PVSA performed 12 weeks post-vasectomy. No significant differences were found ($p < 0.302562$) in early failure rate between the two groups.

Conclusions: These data suggests that surgical technique by referring group has led to an increase in failed vasectomies in the north-west of England. The increase cannot be attributed to reduced interval to analysis post-vasectomy as advised in the 2016 PVSA guidelines. However, further work is needed to evaluate the interval between surgery and analysis before January 2017 to confirm if the reduction of interval from 16 weeks to 12 weeks post-vasectomy has compounded the findings.

P024 The importance of female reproductive tract metabolites for sperm metabolism and motility

Gruszyk Evie; Reynolds Steven

University of Sheffield

Background: The underlying metabolic pathways utilised by sperm could be a contributing factor to poor sperm quality(1). Female reproductive tract (FRT) fluids are rich in metabolites(2, 3). Sperm are exposed to this as they progress towards the ovum leading to a complex interaction between sperm and FRT metabolites(4-6). ^{13}C -Magnetic Resonance Spectroscopy (MRS) can evaluate sperm metabolism(7). This study investigated whether synergistically combining FRT metabolites influenced sperm metabolism, motility and vitality.

Methods: Fifteen sperm aliquots (produced from thirty-one ejaculates washed with 40% Percoll/Earle's balance salt solution density gradients) were incubated for 4 hours at 37°C , 5% CO_2 with antibiotics and either: (i) $^{13}\text{C}_6$ -glucose(control); (ii) $^{13}\text{C}_3$ -L-lactate; (iii) $^{13}\text{C}_3$ -D-lactate; (iv) $^{13}\text{C}_6$ -glucose+ $^{13}\text{C}_3$ -L-lactate; and (v) $^{13}\text{C}_6$ -glucose+ $^{13}\text{C}_3$ -D-lactate. Motility and vitality were assessed before (T0) and after (T4) incubation. ^{13}C -spectra were acquired using a 9.4T MRS spectrometer. Lactate and bicarbonate integrals were normalised to vital sperm concentration.

Results: Progressive motility significantly increased for glucose and glucose+lactate incubations between T0 and T4, however, rapid progressive sperm was not significantly increased. For L- or D-lactate only incubations, progressive motility significantly decreased. Glucose to lactate (glycolysis) was consistently observed by MRS, however, bicarbonate(Krebs cycle) was only observed intermittently. There were no significant differences in normalised lactate or bicarbonate MRS integrals between the substrate incubation. However, ANCOVA analysis of lactate integrals vs vital sperm concentration showed a significant difference in slope between the glucose and glucose+lactate combinations.

Conclusions: Further study is required to understand whether the addition of L/D-lactate to glucose increases the rate of glycolysis and subsequent lactate production, despite no evidence of lactate to pyruvate conversion — in contrast to previous work(7). Investigating the pathways to Krebs cycle bicarbonate production using metabolic inhibitors, the metabolism other known FRT substrates (e.g. pyruvate and glutamate) and determining potential contribution of non-sperm cells to metabolism are also required.

1. Reynolds S, Calvert SJ, Paley MN, Pacey AA. H-1 Magnetic Resonance Spectroscopy of live human sperm. *Molecular Human Reproduction*. 2017;23(7):441-51.
2. Amabebe E, Reynolds S, Stern V, Stafford G, Paley M, Anumba DO. Cervicovaginal Fluid Acetate: A Metabolite Marker of Preterm Birth in Symptomatic Pregnant Women. *Front Med (Lausanne)*. 2016;3:48.
3. Amabebe E, Reynolds S, Stern VL, Parker JL, Stafford GP, Paley MN, et al. Identifying metabolite markers for preterm birth in cervicovaginal fluid by magnetic resonance spectroscopy. *Metabolomics*. 2016;12:67.
4. Lopez-Torres AS, Chirinos M. Modulation of Human Sperm Capacitation by Progesterone, Estradiol, and Luteinizing Hormone. *Reprod Sci*. 2017;24(2):193-201.
5. Aitken RJ, Nixon B. Sperm capacitation: a distant landscape glimpsed but unexplored. *Mol Hum Reprod*. 2013;19(12):785-93.
6. Suarez SS, Pacey AA. Sperm transport in the female reproductive tract. *Hum Reprod Update*. 2006;12(1):23-37.
7. Calvert SJ, Reynolds S, Paley MN, Walters SJ, Pacey AA. Probing human sperm metabolism using C-13-magnetic resonance spectroscopy. *Molecular Human Reproduction*. 2019;25(1):30-41.

P025 A comparison of outcomes of ICSI using cryopreserved sperm

Adedero Samuel¹; Isdale Mari²; Hunter Helen²; Lacey Lauren³; Akhtar Muhammad A²

Background: Sperm cryopreservation is routinely performed in assisted reproduction centres. Both ejaculated sperm and surgically retrieved sperm can be cryopreserved for a variety of indications. Our aim was to review if live birth outcomes varied between those couples who had intracytoplasmic sperm injection (ICSI) with cryopreserved sperm which was ejaculated when compared to sperm that was retrieved surgically.

Methods: Retrospective cohort study from 2014-2017 at an NHS teaching hospital of pregnancy outcomes from cryopreserved sperm comparing 16 couples who had ICSI using cryopreserved ejaculated sperm and 94 couples who had ICSI using cryopreserved surgically retrieved sperm. Data was collected using hospital computer systems/patient notes. Statistical analysis of demographics was performed using Mann Whitney test to identify differences between the two groups. Cumulative live birth rate (CLBR) after three cycles of ICSI was calculated following the method described by Maheshwari et al, 2015 (1).

Results: The median age and interquartile range (IQR) in years of males at the age of sperm cryopreservation was 30.5(29.8-37) and 30.5(25-38) in the surgical retrieved sperm and ejaculated sperm groups respectively (no statistically significant difference, $p=0.2294$). The median age and IQR in years of females at the age of first egg collection was 31.5(28-35) and 31.5(29-38.3) in the surgical retrieved sperm and ejaculated sperm groups respectively (no statistically significant difference, $p=0.8826$). The CLBR after three cycles of ICSI was 50% in ejaculated sperm group and 51.1% in the surgical retrieved sperm group.

Conclusions: Live birth outcomes using cryopreserved sperm were very similar between the two groups. Importantly, female age did not differ between the groups in our cohort. Although our cohort is small, this data can be used to counsel men undergoing surgical sperm retrieval that if sperm is successfully retrieved, their chances of becoming biological fathers is comparable to men who have ejaculated cryopreserved sperm.

1. Maheshwari A, McLernon D, Bhattacharya S. Cumulative live birth rate: time for a consensus? Hum Reprod. 2015;30(12):2703-7.

P026 Comparison of the yield of motile sperm following preparation with different commercial media

Engley Stephanie; Naik Rahul; Richardson Lucy

Herts & Essex Fertility Centre

Aim: Density gradient centrifugation (DGC) is well established for sperm preparation in assisted conception. The ideal sperm preparation technique should yield the highest number of motile sperm. This study compares historically used Pureception Gradients (Sage, USA) and Quinn's Advantage Fertilisation Medium (Sage, USA) at our clinic along with newly available Origio Gradients and Origio Sperm Wash (Origio, Denmark).

Methods: Fifty-five semen samples were divided equally. The percentage motile, total motile sperm and total sperm recovered was assessed using Origio Gradients (OG), Origio Sperm Wash (OSW), Pureception Gradients (PCG) and Quinn's Advantage Fertilisation Medium (FM). The differences in the mean percentages of motile sperm recovered were assessed by repeated measures analysis, $P<0.05$ was considered to be statistically significant.

Results: The percentage of motile sperm recovered following DGC with the Origio Gradients was significantly higher when compared to DGC with the Pureception Gradients. OG and OSW (81.2%) vs PCG and FM (73%). OG and OSW (81.2%) vs PCG and OSW (73.6%). OG and FM (78.7%) vs PCG and FM (73%). OG and FM (78.7%) vs PCG and OSW (73.6%). The recovery for percentage of total motile sperm and total sperm following DGC with the Origio Gradients when compared to the Pureception Gradients did not demonstrate a significant difference.

Conclusion: The results conclude that Origio Gradients yield a higher percent of motile sperm when compared to the Pureception Gradients irrespective of the medium used for the wash step.

P027 A novel approach to sperm selection for ICSI through thermotaxis

Care Fertility

Of the millions of sperm deposited in natural conception, just a thousand may reach the ampulla, and therefore only a fraction are able to fertilise the oocyte(1,2). Despite this number being sufficient naturally, when IVF is performed, a larger density is required. It has been implied that in natural conception sperm are guided to the oocyte and undergo a process of natural selection, positively discriminating sperm of suboptimal quality. Whereas in IVF and ICSI, sperm are selected based upon their motility primarily(2,3).

It is known that a temperature gradient exists along the female reproductive canal(4). The aim of this study is to develop a method using CE marked IVF laboratory products to verify sperm thermotaxis activity and allow their selection for ICSI treatment.

IVF medium (Global Total Fertilisation, LifeGlobal) was organised within the ICSI dish (Vitrolife) using three 'flat' 25uL drops, connected by two 6uL thin 'bridges' of the same media and covered with oil. These dishes were positioned partially on the validated ICSI heated stage for 1hour to establish a temperature gradient(30.2-34.4°C) between the 2 peripheral drops. The control dish remained at room temperature. 50uL of a prepared normal semen sample (WHO(5)) was added to the central drop and left for 1hour to allow sperm migration along the bridges in either direction. Thermotaxis index(TI) was calculated by dividing the number of sperm in the warm drop by the number of sperm in the cooler drop, and for the control dishes number of sperm in the left drop by number of sperm in the right(6).

Results of this pilot study from 8 semen samples showed a TI=2.8 on the gradient dishes compared to a TI=0.8 on the control(P=0.03), demonstrating thermotaxis behaviour. This method of sperm selection can be integrated into standard sperm preparation for ICSI and may help to improve

1. Sakkas, D., Ramalingam, M., Garrido, N. and Barratt, C. (2015). Sperm selection in natural conception: what can we learn from Mother Nature to improve assisted reproduction outcomes?. *Human Reproduction Update*, 21(6), pp.711-726.
2. Bahat, A. and Eisenbach, M. (2006). Sperm thermotaxis. *Molecular and Cellular Endocrinology*, 252(1-2), pp.115-119.
3. Pérez-Cereales, S., Laguna-Barraza, R., de Castro, A., Sánchez-Calabuig, M., Cano-Oliva, E., de Castro-Pita, F., Montoro-Buils, L., Pericuesta, E., Fernández-González, R. and Gutiérrez-Adán, A. (2018). Sperm selection by thermotaxis improves ICSI outcome in mice. *Scientific Reports*, 8(1).
4. Ng K, Mingels R, Morgan H, Macklon N, Cheong Y. In vivo oxygen, temperature and pH dynamics in the female reproductive tract and their importance in human conception: a systematic review. *Human Reproduction Update*. 2017;24(1):15-34.
5. Cooper, T., Noonan, E., von Eckardstein, S., Auger, J., Baker, H., Behre, H., Haugen, T., Kruger, T., Wang, C., Mbizvo, M. and Vogelsong, K. (2009). World Health Organization reference values for human semen characteristics*±. *Human Reproduction Update*, 16(3), pp.231-245.
6. Li, Z., Liu, W., Qiu, T., Xie, L., Chen, W., Liu, R., Lu, Y., Mitchelson, K., Wang, J., Qiao, J. and Cheng, J. (2014). The construction of an interfacial valve-based microfluidic chip for thermotaxis evaluation of human sperm. *Biomicrofluidics*, 8(2), p.024102.

P028 Extracellular vesicles - messengers between the mother and the offspring

Godakumara Kasun¹; Jaakma Ülle²; Salumets Andres³; Fazeli Alireza⁴

¹Tartu University; ²Institute of Veterinary Medicine and Animal Sciences, Estonian University of Life Sciences; ³Competence Center on Health Technologies, Estonia; ⁴Academic Unit of Reproductive and Developmental Medicine, Department of Oncology and Metabolism, University of Sheffield

Background: Embryo maternal communication has long been hypothesized as an essential requirement for a successful embryo implantation. In previous communications, we have reported that several embryonic RNAs are transferred to endometrium and the expression of specific genes in endometrium are altered as a result. In an in vitro system using human choriocarcinoma (JAR) cells as an analogue for the pre-implantation embryo, we have observed that the expression of the exonic region of LINC00478 is down regulated in endometrial cells (1) after co-incubation. Based on this data, we have hypothesized the medium of embryo-maternal cross talk is RNA packaged in extracellular vesicles (EV).

Methodology: JAR spheroids were prepared by rotary shaking of detached cells. Conditioned media of JAR spheroids were collected and subjected to size exclusion chromatography. 18 fractions (fraction size 1 ml) was collected and analyzed using nanoparticle tracking analysis, protein concentration and western blot for EV specific protein markers. Based on the findings, fractions were grouped as pre-EV (fraction 1-5), EV (fraction 6-9) and post-EV (fraction 10-18). 1 x 10⁸ particles from each group were supplemented to 4x10⁵ RL 95-2 cells to investigate the function of each

component of the conditioned media. After a 24h incubation, RNA from RL 95-2 cells were collected and analyzed for the expression of exonic LINC00478 using quantitative PCR with absolute quantitation.

Results: Cells treated with EV fraction exhibited a significant down regulation of LINC00478 compared to the untreated control ($p < 0.001$) while cells treated with pre-EV and post EV fractions were not significantly different from the control ($p > 0.05$).

Conclusions: Results obtained from in vitro model demonstrate that embryonic EVs are integral to embryo-maternal communication.

1. ISEV2019 Abstract Book. Vol. 8, Journal of Extracellular Vesicles. 2019.

P029 The prevalence of chromosomal abnormalities in men with azoospermia and severe oligospermia

Mehboob Neha¹; Dixit Abhijit²; Martin Katherine²; Jayaprakasan Kanna³

¹University of Birmingham; ²University Hospitals of Nottingham; ³University of Nottingham

Background: Men with azoospermia and severe oligospermia are thought to be at an increased risk of chromosomal abnormalities. When subfertile couples with severe male factor are set to undergo intracytoplasmic sperm injection (ICSI), karyotyping is performed in most units. This study aims to estimate the prevalence of chromosomal abnormalities in order to aid in accurate counselling of males that are undergoing karyotype testing.

Methods: This observational study was undertaken in a tertiary referral centre. Online medical records and databases were used to obtain data of all patients with severe oligospermia or azoospermia that underwent karyotype testing between January 2000 to December 2018. The prevalence of chromosomal abnormalities was then calculated amongst the population of eligible patients.

Results: There were a total of 515 patients who had undergone karyotype testing within the study dates. One sample was excluded as it was a duplicate. From the study population of 514: 93.4% (480) samples were karyotyped as normal and 6.6% (34) had chromosomal abnormalities. Most of the chromosomal abnormalities detected were sex chromosome abnormalities (62%; 21/34) of which 17 were XXY and four were XYY abnormalities. The other abnormalities were balanced reciprocal translocation (4/34), Robertsonian translocations (2/34) and mosaic karyotypes (2/34). There were cases of pericentric inversion, paracentric inversion and duplication abnormality.

Conclusion: The prevalence of chromosomal abnormalities in men with severe oligospermia or azoospermia was found to be 6.6%. This data aids in counselling subfertile couples, with severe male factor, prior to embarking on ICSI treatment.

P030 Graphene Oxide as an agent for personalized ARTs

Simões Juliana¹; Ramal-Sanchez Marina¹; Fontana Antonella²; Ramalho-Santos João³; Bernabo Nicola¹; Barboni Barbara¹

¹University of Teramo; ²Università "G. d'Annunzio" Chieti; ³Center for Neurosciences and Cell Biology

Background: In developing countries, an increasing number of couples resort to Assisted Reproductive Technologies (ARTs) with around 50% of all infertility cases being male related and 40% being due exclusively to male causes. Since infertility is a condition that affects not only the health of patients but also over-all life quality, it is of paramount importance to develop techniques that not only improve the success rates of ART but allow for a more personalized and male-directed treatment.

Nanomaterials are the main drivers of personalized medicine. In particular, since the discovery of Graphene in 2004 the range of its possible application in medicine has grown exponentially(1). However, the field of reproduction has yet to explore this opportunity. Recent reports have shown that Graphene Oxide (GO) alone is capable of increasing In vitro Fertilization (IVF) rates in animal models of high predictive value for human medicine(2, 3), due to its ability to

extract cholesterol from the sperm membrane, which enhances capacitation-dependent membrane remodeling(4).

Methods and Results: We incubated normozoospermic semen samples with GO in varying concentrations (0.5; 1; 2; 4µg/mL) in a capacitating media for 3h and evaluated some functional semen parameters, such as motility, tyrosine phosphorylation and acrosome status. Results show that in this range of concentration (which is the most effective in animal models previously tested) GO has no sperm toxicity, presenting no alterations in any of the parameters analysed.

Conclusions: In this work we show that GO in low concentrations can also be used in human ART techniques as it does not induce sperm damage. This suggests that GO is a promising nanomaterial that can be safely used in Andrology and possibly as an agent for the development of more effective and personalized Assisted Reproductive Technologies.

(1) Mao HY, Laurent S, Chen W, Akhavan O, Imani M, Ashkarran AA, et al. Graphene: promises, facts, opportunities, and challenges in nanomedicine. *Chemical reviews*. 2013;113(5):3407-24.

(2) Bernabo N, Fontana A, Sanchez MR, Valbonetti L, Capacchietti G, Zappacosta R, et al. Graphene oxide affects in vitro fertilization outcome by interacting with sperm membrane in an animal model. *Carbon*. 2018;129:428-37.

(3) Ramal Sanchez M, Valbonetti L, Tsikis G, Dubuisson F, Blache M-C, Labas V, et al. Graphene oxide: A glimmer of hope for Assisted Reproductive Technology. *Carbon*. 2019;150.

(4) Bernabo N, Machado-Simoes J, Valbonetti L, Ramal-Sanchez M, Capacchietti G, Fontana A, et al. Graphene Oxide increases mammalian spermatozoa fertilizing ability by extracting cholesterol from their membranes and promoting capacitation. *Scientific reports*. 2019;9(1):8155.

P031 The frozen donor oocyte service: Implementing optimisation strategies to improve patient outcomes

McEvoy Keith; Panagiotopoulou Nikoletta

Manchester Fertility

Purpose/background/objectives: Review of Human Fertilisation and Embryology Authority (HFEA) data for 2017 identified that almost 13% of ART cycles utilized donor oocytes and/ or sperm (1). In the UK, oocyte donor screening guidelines are clearly outlined by the HFEA (2). Nevertheless, the lack of a quarantine period when fresh donated-oocytes are used has the potential to expose recipients to communicable diseases. Advances in cryobiology have led to the successful establishment of oocyte cryo-banks that eliminate the above risk, while also providing additional patient benefits. The purpose of this review is to highlight how continual review and optimisation of an oocyte banking service is key to improving outcomes.

Methods: A total of 208 oocyte warm recipient cycles over a three-year period were reviewed and data was analysed focusing on assessing oocyte survival and clinical pregnancy rates. During this period, there were 3 substantial changes to the service: 1) Defining optimal number of vitrified oocyte, 2) Laboratory procedure standardisation, and 3) Oocyte vitrification/warming protocol optimisation.

Results: A significant increase in clinical pregnancy per recipient oocyte warm was observed when patients were allocated 6 or more oocytes as opposed to a minimum of 4 (40.5% vs 24.5%; P=0.014). Utilisation of spare embryos for freezing has increased from 9.86% (36/365 embryos) to 13.9% (93/700 embryos) following changes. Oocyte survival was 72% (480/667 oocytes) increasing to 78.5% (661/842 oocytes) following phases of optimisation suggesting a trend of increasing survival rate.

Conclusions: Increasing the minimum oocyte number improved recipients' clinical pregnancy chances while offering them an increased chance of freezing surplus embryos. In addition, optimisation of laboratory processes improved oocyte survival rates, even though long-term data are still pending. Next steps will involve looking into new carriers or media, or a combination of both, preferably in a randomised trial setting.

References: 1. HFEA 2019. Fertility treatment: trends and figures [online]. Available at: <https://www.hfea.gov.uk/about-us/publications/research-and-data>. Accessed on 1/8/2019 2. HFEA 2019. Code of practice. Edition 9.0 [online]. Available at: <https://www.hfea.gov.uk/media/2793/2019-01-03-code-of-practice-9th-edition-v2.pdf>. Accessed on 1/8/2019

P032 Retrospective audit of a sperm donor service: total motile sperm count and pregnancy outcome

Asaciovaite Viktorija¹; Ferguson Elizabeth¹; Peddie Valerie²

¹University of Aberdeen; ²NHS

Introduction: Sperm donor (SD) Intra-Uterine Insemination (IUI) services were suspended at Aberdeen Fertility Centre between 10th November 2018 - 22nd January 2018 in response to radical drop in Clinical Pregnancy Rate (CPR) from 20% to 8%. Results were compared with the national average which was 17% for women under 35 and 14% for women aged between 35 and 37. A retrospective audit of our results between February 2018 and February 2019, showed 100% increase in CPR (16%).

Method: We increased the requirements for total minimal count of motile sperm from 1 to 4 million in cases where extra sperm straws could be used. Sperm samples were prepared by thawing, washing by centrifugation at 1300rpm for 10 minutes and re-suspended for analysis. We also audited patient response: confirmation of ovulation in natural cycles and follicular response to ovulation induction in stimulated cycles together with consumables (IUI catheter/syringes) used. We changed IUI catheter to that capable of delivering lower sperm volumes. Data was analysed from our local database.

Results: 72 patients underwent 176 donor IUI cycles. The overall CPR was 16%. Data was split further by dividing the IUI cycles into different groups based on the total motile count (Group 1: 2.5 to 3.9 (n=22); group 2: 4.0 to 7.9 million (n=88), group 3: 8.0 to 11.9 million (n=46), group 4: 12.0+ million (n=20)). The results showed no significant differences (Chi square test, p=0.55) in the CPR between the groups. There were no significant differences in patient ages (ANOVA, p=0.67) and previous number of cycles (ANOVA, p=0.55) across the four groups.

Conclusion: Revision of our sperm donor clinical practice resulted in a significant increase (100%) in CPR. We found that total motile sperm count had no influence on CPR.

P033 Predictive factors of total fertilisation failure in conventional IVF

Hogg Emma¹; Hulme Davina²; Cutting Rachel²

¹The University of Sheffield, UK; ²Jessop Fertility, UK

Background and Aims: Approximately 5% of conventional IVF cycles result in total fertilisation failure (TFF), where no oocytes successfully fertilise. This may be reduced if at-risk patients could be identified and offered ICSI, a treatment not necessarily suitable for all patients, hence the need to identify those that could benefit. This study aimed to identify patient and/or cycle factors that could predict which patients are at higher risk of TFF with conventional IVF.

Methods: This retrospective study analysed all conventional IVF cycles resulting in TFF at a single clinic between January 2008 and December 2018 (n=151). 17 factors were recorded about each patient/cycle and compared to a control group (n=151), consisting of randomly selected patients that achieved a high fertilisation rate (>60%) with conventional IVF over the same period. Significantly different factors were used as independent variables in binary logistic regression and, for each factor found to be associated with TFF, ROC curve analysis was carried out to identify ranges of values that indicate at-risk patients.

Results: 12/17 factors were significantly (p<0.05) different between the TFF and high fertilisation groups. Five factors had a significant association with TFF and ranges of values that indicate a greater risk of TFF were identified for each: number of previous pregnancies (=0), percentage of A-graded sperm before (<17.5%) and after (<72.5%) semen preparation, peak serum oestradiol (<6422pmol/L) and peak oestradiol per oocyte collected (>878pmol/L). If at least 60% of these factors fall within their TFF range, the patient would be at high-risk of TFF and may benefit from ICSI treatment.

Conclusion: Patients with no previous pregnancies, lower percentages of A-graded sperm before and after semen preparation, lower peak serum oestradiol and higher peak oestradiol per oocyte have an increased risk of TFF. Future work should validate the accuracy and predictive value of these findings.

P034 Which donor sperm bank will give the best chance of success? -- A multicentre, retrospective study

Thirlby-Moore Sarah¹; Armstrong Ellen¹; Campbell Alison²

¹Care Fertility Birmingham; ²Care Fertility

Aim: To ascertain if there is a difference between the outcomes of standard IVF-donor sperm cycles, based on the bank.

Method: Retrospective analysis of 194-cycles (2013-2018, 8-clinics) divided into 5-donor sperm bank groups (A-E, n=12,13,61,94 and 13, respectively), comparable on patient age, number of oocytes collected, maturity, number of embryos transferred and culture conditions. Outcomes measured were normal fertilisation, cleavage, attainment of embryo transfer (ET), embryo utilisation, average day of ET, biochemical and clinical pregnancy (CP) rates per treatment started and per ET.

Results: There is a significant difference in fertilisation ($p=0.0353$), day of ET ($p=0.0309$), embryo utilisation ($p=0.00517$), and biochemical and CP rates/treatment started ($p=0.0180$ and $p=0.0211$, respectively). No significant difference is found in cleavage ($p=0.963$), number of ETs performed ($p=0.0506$), or biochemical and CP rates/ET ($p=0.0635$ and $p=0.0692$, respectively). Bank-B, despite the lowest fertilisation rate of 65.7%, offers the greatest chance of success/treatment started with a CP rate of 76.9% and the highest embryo utilisation rate of 59.4%. Conversely, bank-D gives the lowest with a fertilisation rate of 68.6%, a lower chance of attaining ET at 79.8%, and ETs tending towards cleavage stage. This agrees with the reduced embryo utilisation rate of 39.9% and a CP rate/treatment started of 27.7%. Bank-C offers the second highest CP rate/treatment started of 44.3%, thus ranking second. Banks-A and E achieve similar CP rates/treatment started of 33.3% and 30.8%, respectively, however bank-A delivers the lowest embryo utilisation rate of 37.2%, placing it fourth.

Conclusions: The decision of which donor sperm bank to choose is an overwhelming one for patients. With a range to choose from, the real question is, 'which one will give me the best chance?'. With statistical evidence, we can now begin to answer this. Further study is advised with larger patient populations, and live birth outcomes included.

P035 Evolving policies and procedures for successful operation of a donor sperm bank

Ottey Michelle

Fairfax Cryobank

More than ever families are being created using donor sperm¹; it is the responsibility of Cryobanks to ensure regulatory compliance, increased safety through infectious and genetic disease testing, accuracy in record keeping, and the highest quality standards in the preparation of donor sperm samples. The key to successful operation of a Cryobank is an effective, applicable, and clear Standard Operating Procedure Manual (SOPM) that ensures compliance with domestic and international regulations while building a pool of donors that meet the needs of the prospective recipient population. The SOPM addresses both external regulatory requirements as well as internal operational directives that cover all aspects of the processes. Monitoring SOPM success is achieved through regulatory inspections, internal quality assurance and quality control (QA/QC), analyses of sales and distribution trends, and periodic surveys of donor sperm recipients. Opt-in survey results over time demonstrate that more women are using donor sperm because there is no partner to provide sperm. A 2018 survey identified that 81.05% of those who indicated a purchase of donor sperm noted "no male partner", 16.86% "male factor infertility", and 2.09% "male genetic risk". Though we have observed an increase over time in the number of purchasers of ID donor sperm, there remains a population that choose or in the case of some international markets are required to use NonID release donors. Data collected from surveys and sales guides the Cryobank to adjust recruiting strategies, production processes, and policies. Close attention to the changing landscape of parentage laws and gamete donor regulations are critically important. As regulations and laws evolve over time, it is the important for the Cryobank to update policies and procedures to evolve as well; this ensures that the needs of the recipients and the donor conceived individuals are being met.

1. Estimates of donated sperm use in the United States: National Survey of Family Growth 1995-2017 Rachel Arocho, Ph.D., Elizabeth B. Lozano, M.A., M.S., and Carolyn T. Halpern, Ph.D. Carolina Population Center and Department of Maternal and Child Health, UNC Gillings School of Global Public

P036 Clinical audit of the performance of Herts and Essex Fertility Centre's donor sperm bank

Fryer Hayley; Drinkwater Abbie; Naik Rahul; Richardson Lucy

Herts and Essex Fertility Centre

Background: Herts and Essex Fertility Centre (HEFC) provide an in-house donor sperm (IHDS) bank which patients are able to use as an alternative to external donor banks. Recent donor screening guidelines advice clinics to audit donor performance to ensure suitable donors are being recruited (1). Consequently, this study aimed to assess the clinical outcomes of patients using IHDS compared to external donor sperm (EDS).

Methodology: A retrospective cohort study was conducted on patients attending HEFC for fresh ICSI cycles using IHDS (n=67) and EDS (n=36) between August 2016 and August 2018. Patients were categorised by age (18-34, 35-39 and ≥40) and baseline characteristics including BMI, smoking habits and female fertility issues (ovulatory, tubal or uterine disorders) were recorded during initial consultation. In instances where donor oocytes were used, the baseline characteristics of the donor were reported. Cycle outcomes including pregnancy test outcome (positive or negative), pregnancy type (clinical or biochemical), and birth outcome (live birth (LB) or miscarriage) were compared. An unpaired T-Test and Pearson's Chi Squared assessed statistical differences between groups. Significance was accepted at $P < 0.05$.

Results: No significant difference was found in pregnancy test outcomes, pregnancy type or birth outcomes between those using IHDS and EDS for any age group. Patients using IHDS were significantly more likely to smoke than those using EDS ($P = 0.009$). No significant difference was found in any other baseline characteristic examined.

Conclusions: These results support the conclusion that the donors provided by HEFC are suitable for use, and are as likely to result in LB as sperm provided by an external bank. This means the patients attending HEFC have the option of selecting from high quality in-house sperm donors, without the additional costs incurred when sourcing sperm from an external bank.

(1) Clarke H, Harrison S, Perez MJ, Kirkman-Brown J. UK guidelines for the medical and laboratory procurement and use of sperm, oocyte and embryo donors (2019). Human Fertility. 2019. Jun 6:1-13.

P037 Experience with a large pan-ethnic, carrier screening panel on a sperm donor population; lessons for the informed consent process

Callum Pamela; Baldwin Kara

California Cryobank

Expanded carrier screening (ECS) is becoming a routine evaluation on gamete donor applicants. Experience with screening on sperm donor applicants illustrate that testing can reveal significant health implications for the individuals being tested, in addition to routine reproductive risks. **METHODS:** Carrier screening for 260 autosomal recessive (AR) disorders was performed on 472 sperm donor applicants between July 2017 and August 2019. **RESULTS:** Four men were identified to have two variants for autosomal recessive diseases for which they may develop symptoms. Four other men carry mutations in genes for which their heterozygote status confers an increased risk for cancer and other disorders in the men themselves. The mutations are transmitted in a dominant manner and each offspring who inherits the mutation (50%) would also have increased disease risks. **DISCUSSION:** Inclusion of hundreds of genes on ECS panels prohibits detailed pre-test patient counseling about the natural history of each disorder and all of the possible results. As illustrated, compound heterozygotes may not always be at-risk for symptoms depending upon the specific mutations detected, but in some cases these findings can be predict significant disease risks in these individuals. Reproductive patients may be unprepared to manage such information. Ordering providers help to prepare patients for such possibilities by educating them about the spectrum of known risks, including those described above, as well of the possibility of other, yet unknown risks from genetic testing as part of the informed process. All donor applicants

described above were excluded from participation in the sperm donor program at this time because recipients and their providers are only just becoming familiar with the use of large carrier screening panels and the risks posed by the findings reported here require more detailed counseling than average carrier results and such counseling may not be routinely available when selecting a donor.

P038 Exploration of the clinical scientist's experience of carrier matching for donor selection in the IVF clinic

Shaikly Valerie; Ahuja Sapna; Taranissi Mohamed

ARGC

Purpose: To gain an improved understanding of patient requirements and make recommendations for service development as carrier testing in gamete donors shifts to become mainstream.

Methods: This study reports on the experience at the ARGC, London. 15 Patients wishing to purchase samples from donors for which extended carrier screening results showed a positive carrier status, for one or more recessive conditions, were provided with the opportunity of a telephone dialogue with a clinical scientist. This included the meaning of the carrier status, risk estimates and options. Patients were given the opportunity to review the consultation report and were supported through their subsequent decision making. The patient choices made were then evaluated to identify common themes in the patient decision pathway.

Results:

Pathway 1 - to select an alternative donor that has not had carrier screening.

Pathway 2 - to order the samples for a treatment cycle in the short term but additionally opt to have carrier testing to 'check' in the long term.

Pathway 3 - to have carrier screening to ensure there is no carrier match. However, panels available commercially also had the potential to identify a carrier status that was not tested in the panel used for the donor screening.

Pathway 4 - to not to have carrier screening, sign a declaration to confirm understanding of the risks and proceed with using the donor.

Conclusion and recommendations: The pathways identified from lessons learnt have value for service refinement in licenced clinics. Potential match yield in carrier panels should be considered. Patient choice of a donor with or without carrier matching is empowering; it is important that clinics develop tools to deliver and support patients to make informed decisions for donor selection.

P039 The outcomes in pregnancies from the use of donor sperm compared with those resulting from partner sperm. A systematic review and meta-analysis

Allen Christopher¹; Marconi Nicola²; McLernon David¹; Bhattacharya Sohinee¹; Maheshwari Abha²

¹University of Aberdeen; ²Aberdeen Fertility Centre

Introduction: Registry data from HFEA show an increase of 40% in IUI and 377% in IVF cases using donor sperm.

Methods: We performed a systematic review and meta-analysis on the pregnancy and perinatal outcomes of pregnancies conceived with donor sperm. Comparing them to conceptions with partner's sperm to assess whether outcomes of these two groups differed. The Downs and Black tool was used for quality assessment.

Results: 37 studies were included in the review, 36 in the meta-analysis. For pregnancies conceived with donor sperm (versus partner sperm) there was an increase in the odds (95% CI) of hypertensive disorders of pregnancy, 1.50 (1.20 -- 1.88), pre-eclampsia, 1.49 (1.05 -- 2.09), small for gestational age (SGA) 1.44 (1.15 -- 1.80) but a reduced risk of ectopic pregnancy 0.66 (0.46 -- 0.96). There was no difference in the odds (95% CI) of miscarriage, 0.95 (0.78 - 1.16), gestational diabetes, 1.49 (0.62 -- 3.63), placental abruption: 0.65 (0.04 - 10.56), placenta previa, 1.19 (0.64 - 2.22), preterm birth, 1.01 (0.89 - 1.14), low birth weight, 0.97 (0.80 - 1.18), large for gestational age, 1.02 (0.83 - 1.24),

stillbirth, 1.23 (0.97 -- 1.58), neonatal death, 0.78 (0.36 - 1.73), and congenital anomaly, 1.11 (0.94 - 1.32). All included studies were based on observational data and varied in their quality and risk of bias.

Conclusion: While largely data is reassuring, we must be alert to the increased risk of hypertensive disorders of pregnancy and SGA in pregnancies as a result of donor sperm. Appropriate counselling and screening must be put in place as this may have long term implications. This has repercussions for clinical service and users as this may involve increased antenatal input. Long term outcomes in these pregnancies should be monitored as part of routine data collection due to the associated risks.

P040 Caring for egg donors: analysing experiences of 454 women donating eggs to a licensed cryobank in the UK

Wolska Marta; Arian-Shad Mimi; Ahuja Kamal; Macklon Nick

London Egg Bank, London Women's Clinic

Objectives: For many years egg donation programmes located overseas have been an attractive source of donor eggs for British women. However, since 2012, changes in Human Fertilisation Embryology Authority regulations have theoretically made it possible for licensed clinics to attempt to gain self-sufficiency in this area. In this first ever study of UK donors, we report on their experiences of donating eggs at a licensed cryobank in UK.

Methods: The study presents the survey results from 454 egg donors who consecutively participated in our programme during 2016 -- 2018. Invitations were sent via Survey Monkey to 431 women who had completed egg collection and to 23 who were declined acceptance on account of inadequate compliance with requirements. 16 questions were asked under the following 4 headings: Relationships with the egg bank team; Outcome of fertility assessment; Experience of egg collection; Experience post egg collection and follow up care. The qualitative and quantitative responses received within 4 weeks were analysed.

Results: Overall 30% of those approached (131/454), responded; In each category, 91 - 99% approved of their overall experience at the clinic. All were happy to have participated in helping others. Nearly three quarters (73%) have either considered or completed a second donation. Over 90% stated that they would like to receive a fertility health check-up within 2 years. Twenty six women also registered unfavourable experiences, mostly related to lack of sensitivity experienced post egg collection. The policies and practices of the clinic are being altered to take account of the strong recommendations from these responses. Addressing these issues can help increase the availability of donor eggs and opportunities for donor egg treatment within the UK.

P041 The evolution of surrogacy: a legal and clinical perspective

Williams Eleri¹; Steyn Francesca²

¹Hill Dickinson; ²The Centre for Reproductive and Genetic Health

Whilst surrogacy was once a taboo subject to many, especially Parliamentarians, it is now an increasingly popular form of assisted reproduction, with surrogacy law reform finally on the political agenda. This presentation will consider the evolution of surrogacy from both a clinical and legal perspective, and how the practice is likely to evolve in the future, both in the UK and internationally. Clinical: a survey will be sent to UK clinics which provide surrogacy treatment, to collect empirical data about whether there are any particular growing trends and clinical outcomes in this area, and to establish whether surrogacy is becoming more prevalent. There are many considerations for a clinic to take into account when treating a surrogate, including the wishes of all those involved, the surrogate's wellbeing and the welfare of the child. This is only going to become more prevalent as the use of surrogacy increases. Legal: the regulation of surrogacy is a complex area of law, in terms of both the surrogacy arrangement the parties enter into, and legal parenthood. The key areas of the current law will be discussed, with reference to controversial case law. The Law Commission's proposals set out in their recent consultation paper on law reform will also be reviewed. The presentation will compare the UK law with international law, including those countries where the laws are particularly lenient or strict, and consider whether the Law Commission's proposals for law reform will likely deter intended parents from seeking treatment abroad (as they hope), in favour of entering surrogacy arrangements in the UK.

P042 Think before you test. One clinic's experience of ancestry DNA testing and donor conceived children

Freeman Charlene; Thomas Mo; Blower Jane; Gelbya Tarek

Leicester Fertility Centre

The advent of genetic testing has led to a rise in successful criminal prosecutions and knowledge about potential heritable disorders. It is used within the justice system and, with large project collaborations within the NHS such as the 100,000 Genome Project, and will become main stream within the medical field; personalising treatment for individual genotypes. Direct to consumer DNA testing is relatively inexpensive and easily available via online websites, many people are using testing to learn more about their genealogy and ancestral history. However this has led to reports of individuals discovering their heritage isn't what they expected, including cases of non-paternity. The ethics surrounding this area of science are complex; many of the sites offering DNA testing for this reason have only brief statements about possible unexpected outcomes with little to no follow up procedures in place. Users have the option to link to other profiles within the site with similar DNA; so in addition to finding more information about what percentage DNA they have in common with known relations such as parents and siblings who are also using the site(s), they can also discover people with a mutual relative and look at the percentage DNA shared. Whilst this helps create a family tree and discover our ancestors it can also make it easier for people to track down previously unknown biological relations. Early in 2019 one of our first anonymous sperm donors from the 1980's attended our clinic after being contacted by one of his donor conceived children via an ancestry DNA website. This is the story of our experience.

P043 The meaning of social support to well-being of couples undergoing infertility treatment

Malina Alicja; Suwalska-Barancewicz Dorota

Kazimierz Wielki University, Faculty of Pedagogy and Psychology

This study aims to explore the relations between social support and psychological well-being of infertile couples. The study involved 50 heterosexual couples qualified for IVF. Participants filled questionnaires regarding social support (The Social Support Questionnaire (K-Wspo in Polish adaptation of Juczyński) and well-being (Psychological Well-Being Scale by Karaś, Ciecuch). The statistical analysis indicates that significant correlations can be found between social support and well-being of women and men undergoing IVF. The study also looked at couples medical history and family background as mediators in this relationship.

P044 Stress and resilience in relations to psychological well-being of couples undergoing infertility treatment

Malina Alicja¹; Głogiewicz Małgorzata²; Piotrowski Jakub³

¹Kazimierz Wielki University, Faculty of Pedagogy and Psychology; ²Collegium Medicum UMK; ³Nicolaus Copernicus University, Faculty of Biology and Environment Protection, Department of Immunology

This study aims to explore the relations between psychological resilience, reaction to stress (somatic and emotional) and subjective well-being in the context of infertile couple's family background and their medical history. In Poland, where the study was conducted, the acceptance of ART (Assisted Reproductive Technology) methods is still lower than in western European 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 problem for many Polish couples. 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 divided between the experimental and control group. The first stage of the research procedure was carried out with 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. The other half of participants- couples undergoing infertility treatment were subject to a supportive social interaction. The interaction was conducted in 5 separate groups- 5 couples each 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. An information about the history of infertility treatment was also collected. The couples filled psychological questionnaires regarding: perceived stress (Perceived Stress Scale - PSS-10), resilience (Lifespan Individual Resilience Scale (pl)), social support (The Social Support Questionnaire (K-Wspo), and well-being (Psychological Well-Being Scale) The statistical analysis indicates a significant relationship between cortisol decrease and psychological factors as well as the history of infertility treatment of couples undergoing IVF.

P045 Obstetrics and gynaecology doctors views and beliefs regarding social and medically-indicated egg freezing

Farahani Linda; Gadd Gabriella; Oke Tomilola; Mittal Monica; Webber Lisa; Salim Rehan; Rai Raj

Imperial College Healthcare NHS Trust

Background: There is a trend towards delaying childbirth in the UK(1). Egg freezing is a method of preserving fertility, whether for medical or social reasons. Misinformation/lack of information will impact the choices women make, and the controversial nature of egg freezing may create obstacles to those seeking help. The HFEA published recent guidance on egg freezing(2), and this study aimed to assess if access to clear information would alter decision-making.

Methods: A survey study was conducted between October and December 2018. A 20-question questionnaire was sent to 136 obstetrics and gynaecology (O&G) doctors. The questionnaire contained a hyperlink directing respondents to the HFEA guideline, and were asked to read this prior to completion

Results: The response rate was 79%. 74% of respondents were aged 30-39. 60% of respondents had children. Of the 40% who did not have children, 89% would like/have liked to have them. 2.5% of respondents had frozen their eggs before completing the questionnaire. 24% of respondents agreed that with this information they would have elected to freeze eggs earlier. This difference is statistically significant($p < 0.0001$). 5% of respondents regretted not freezing their eggs, and this increased to 33% in respondents over the age of 35. The five major themes that emerged were; * Positive option for women * Cost/safety concerns * Ethical/moral concerns such as coercion, or false sense of security * Justified only when medically-indicated * Education on this subject vital to help counsel others The proportion of positive and negative comments was not significantly different ($n=45$ vs 39 , $p=0.48$).

Conclusions: Despite working within O&G with better access to this information, a significant proportion reported regret and would have elected for egg-freezing. Egg-freezing remains a controversial issue as demonstrated by the emerging themes. Accurate, unbiased and accessible information about this important health issue will enable women to make informed choices

1. Births in England and Wales: 2018 (Internet). Office for National Statistics. 2019 (cited 16 September 2019). Available from <https://www.ons.gov.uk/peoplepopulationandcommunity/birthsdeathsandmarriages/livebirths/bulletins/birthsummarytablesenglandandwales/2018>
2. Egg freezing (Internet). Human Embryology and Fertilisation Authority. 2018 (cited 16 September 2019). Available from <https://www.hfea.gov.uk/treatments/fertility-preservation/egg-freezing/>

P046 Clinical twin pregnancy reported after delayed ICSI 24 hours post oocyte retrieval

Rhodes Sam¹; Nice Lynne¹; Kazem Rahnuma¹; Campbell Alison²

¹CARE Northampton; ²CARE

Aims/Objectives: To report the results of a case study following delayed intracytoplasmic sperm injection (ICSI) which achieved clinical twin pregnancy.

Content of presentation: No metaphase 2 (MII) oocytes on day of oocyte retrieval (TVOR). Oocytes matured overnight and injected. Day 6 single embryo transfer (sET) achieved ongoing clinical twin pregnancy.

Relevance/Impact: Information for embryologists for future cases to determine best treatment plan and outcome for patients. Review of best practice for observations and culture of metaphase I (MI) oocytes.

Outcome: Patient protocol Buserelin and Menopur 150iu 12 days with Metformin. Day 9 of stimulation 10 follicles measured 17-22mm and TVOR performed 36 hours post hCG trigger. Post denudation and oocyte assessment every few hours 3 MI and 4 germinal vesicle (GV) oocytes remained in non IVM culture media with no FSH/LH supplementation overnight. 24 hours post TVOR 4 MII oocytes suitable for ICSI. 3 GVs were disposed (overnight maturity 57%). 100% fertilisation rate (4/4) confirmed. Embryo development and utilisation rate reported as good; sET HB2,2 day 6 and cryopreservation of HB2,2. Remaining embryos; early blastocyst and compacting embryo disposed. Positive pregnancy test, 2 foetal hearts and monochorionic diamniotic twin pregnancy confirmed by 6 week ultrasound scan.

Discussion: There is variation within the literature surrounding delayed ICSI and patient outcomes and sample sizes are low. Delayed ICSI has been performed when no MII oocytes available on day of TVOR or following failed IVF fertilisation. The consensus is delayed ICSI results in reduced fertilisation rate, poorer embryo quality, lower implantation and clinical pregnancy rates compared to standard patients. This case study reports a higher overnight maturity rate and fertilisation rate with positive clinical outcome.

Conclusion: Delayed ICSI reportedly achieves poorer outcomes. However, performing delayed ICSI could give specific patients opportunity for a successful outcome where they would otherwise have a cancelled cycle.

P047 Should a reactive oxygen species (ROS) measurement be part of routine semen analysis?

Pool Georgina; Rogers Shaun; Florek Agnieszka

GENNET City Fertility

Background: Semen analysis has classically been used to determine male fertility. However, measuring standard parameters alone cannot accurately predict infertility, as between 6-27% of men with normal semen parameters are infertile [1]. Current semen analyses are unable to detect subtle sperm dysfunction at a molecular level, which may be contributing to cases described as unexplained infertility. Excessive production of ROS leads to oxidative stress which can negatively influence sperm motility, vitality and impair capacitation and sperm-oocyte fusion [2]. Elevated ROS levels are associated with an increased time to natural conception, reduced blastocyst development and recurrent miscarriage [3]. We aimed to determine whether ROS measurement can provide a benefit as part of routine semen analysis.

Methods: A measurement was performed in 116 men undergoing routine semen analysis using the Mioxsys reader (Aytu BioScience, USA), which provides an integrated measure of all oxidants and antioxidants within a semen sample. The reading derived is a standard oxidative-reductive potential (sORP) which was normalised to sperm concentration and classified as normal if below a threshold of 1.45 mV/106. Routine semen parameters were also measured during the analysis including leukocyte presence, to establish if leukocytospermia is the root cause for excessive ROS.

Results: Concentration and progressive motility were reduced in the men with abnormal sORP ($P < 0.05$). 86% of men with abnormal semen parameters had abnormal sORP levels. 47/116 men had abnormal ROS readings, yet 60% of these had normal semen parameters. Therefore, 25% of men in this study would be classified as normal but had abnormal ROS levels.

Conclusions: These findings demonstrate ROS as a useful predictor of abnormal semen samples. High levels of ROS in a seemingly normal sample may be contributing to the cases of unexplained infertility.

[1] Kumar N, Singh AK. Trends of male factor infertility, an important cause of infertility: A review of literature. Journal of human reproductive sciences. 2015 Oct;8(4):191.

[2] Aitken RJ, Baker MA. Oxidative stress, sperm survival and fertility control. Molecular and cellular endocrinology. 2006 May 16;250(1-2):66-9.

[3] Agarwal A, Sharma R, Roychoudhury S, Du Plessis S, Sabanegh E. MIOXSYS: a novel method of measuring oxidation reduction potential in semen and seminal plasma. Fertility and sterility. 2016 Sep 1;106(3):566-73.

P048: An overview of the management and pathway of a nurse led recurrent pregnancy loss service

Capotescu Anca; Maison Chantelle; Seshadri Srividya; Steyn Francesca; Saab Wael; Serhal Paul

Having a miscarriage can be very distressing especially after undergoing fertility treatment. To have several miscarriages can be devastating. The Miscarriage Association explains that recurrent miscarriage affects 1 in 100 (1%) couples trying to have a baby. Women who have supportive care from the beginning of a pregnancy have a better chance of a successful birth according to a small study. In order to support these groups of patients, in 2019 a nurse led recurrent pregnancy loss programme has been implemented where ongoing specialist nursing support and a dedicated link nurse team have been provided to the patient from the start of treatment all the way through to early pregnancy. Specialist Fertility Nurses are well placed to provide the support and meet both the clinical and emotional needs of patients experiencing RPL as they often have frequent contact with the patients and are fully aware of the patients medical history. The RCN 2019 explain that nurses can provide good mental health by building good relationships with patients. This service is provided to both male and female patients and although the number of patients enrolled in the current programme are currently small, feedback has shown that the nurse led input has been positive and patients feel supported. It is also important to understand that the provision of nurse led support for couples experiencing RPL is of extreme importance and feelings of failure, trauma and devastation are normal, however some couples will require further referral for professional counselling or support.

ESHRE, 2017. Recurrent Pregnancy Loss. [online] Available at: <https://www.eshre.eu/Guidelines-and-Legal/Guidelines/Recurrent-pregnancy-loss.aspx> [Accessed 15 August 2019] Miscarriage Association, 2019. Recurrent Miscarriage. [online] Available at: <https://www.miscarriageassociation.org.uk/information/miscarriage/> [Accessed 15 August 2019] RCN, 2019. Mental Health. [online] Available at: <https://www.rcn.org.uk/clinical-topics/mental-health> [Accessed 15 August 2019] Stray-Pedersen B, Stray-Pedersen S. Etiologic factors and subsequent reproductive performance in 195 couples with a prior history of habitual abortion. Am J Obstet Gynecol 1984;148: 140-146

P049 Unexplained infertility is increasing over time and across different age groups

Louzada Julio; Jacques Celine; Barkaoui Samya; Beaumont Lucy; Kotrotsou Mara; Noublanche Caroline; Hickman Cristina

Apricity

Introduction: According to the HFEA database, how has the type of infertility of patients treated in the UK changed over time and across different age groups?

Methods: Population based cohort study using all cycles from the HFEA register (2000 to 2016). All cycles from female age cohorts 18-34(n=287062cycles), 35-37(n=153389cycles), 38-39(n=96561cycles), 40-42(n=80720cycles), 43-44 (n=19647cycles) and 45-50 (n=5632cycles) years were included. Type of infertility categorised as Unexplained, Male, Female, Both. Female infertility sub-categorised as Unexplained, Ovulation Disorder, Endometriosis, Tubal Disease.

Results: From 2000 to 2016, the proportion of unexplained infertility increased from 7.7% to 22.5%. Female infertility has largely stayed unchanged over time (44%), whilst male infertility (36.0% to 25.9%) and Both (15.1% to 7.4%) have marginally reduced. For the same period, within female infertility, female unexplained infertility has also increased from 33.6% to 50.5%, whilst ovulatory disorder (21%) and endometriosis (10%) have remained unchanged, and tubal infertility has reduced from 37.1% to 18.4% (p<0.001). The proportion of male factor infertility decreased with female age (36,31,29,25,20,14% for each of the female age cohorts respectively from <35 to 45-50), as did the proportion of tubal factor within female infertility (31,29,27,24,20,16%, respectively).

Conclusion: Reduction in male factor infertility with increasing female age and increased unexplained infertility given improvements in fertility diagnostics over the same period are both contrary to what is expected and may be due to data entry error and/or lack of focus on male infertility diagnostics by UK clinics. Further studies are required to identify how this data can be collected accurately, and/or whether clinics are spending adequate resources in diagnosis. Standards on infertility diagnostics are needed.

P050 The storage and disposal of embryos and gametes in the UK: regulations in need of review

Lawford Davies James; Williams Eleri

Hill Dickinson LLP

The regulations governing the storage of embryos and gametes in the UK are in need of review. This is illustrated by three concurrent problems which affect many, if not all, UK clinics:

First, many UK clinics currently face difficulties relating to material stored beyond its permitted statutory storage period, particularly where the required medical evidence in support of extended storage has not been obtained within the requisite period. In many cases, absent a court order, the material will have to be destroyed.

Second, a significant number of women who froze their eggs for non-medical reasons have now reached (or are approaching) the end of the permitted ten-year storage limit and are confronted with the choice between the disposal of their eggs, using them before they are ready to do so, or seeking to export their eggs abroad.

Third, a number of recent cases have illustrated the difficulty of applying the existing regulations to complex scenarios, particularly where a patient dies unexpectedly or without completing the required consent documentation.

This paper will review the existing regulations and how they operate in practice in these three areas, including recent case law. It will then consider how the regulations could be revised to better reflect the interests of patients and the clinics that store their embryos and gametes. It will also consider the Storage Period for Gametes Bill currently before the House of Lords, and alternative approaches adopted in other jurisdictions.

P051 Towards a clear referral pathway for fertility preservation in survivors of childhood cancer

Hartigan Lucia¹; Glover Louise²; Groarke Helen²; Wingfield Mary¹

¹Merrion Fertility Clinic and National Maternity Hospital; ²Merrion Fertility Clinic

Context: Long-term survival is now expected in 80% of children, adolescents and young adults (CAYA) diagnosed with cancer. However many survivors experience 'late effects' of treatment, including loss of fertility.(1)

Objective: To assess (1) knowledge and attitudes of Irish healthcare professionals (HCP) regarding fertility preservation (FP) for CAYA with cancer and (2) interest in fertility assessment among young female survivors. Methods: (1) Four groups of HCP were surveyed: Doctors/nurses at the National Paediatric Haematology and Oncology Centre, generalist paediatricians, trainees in obstetrics/gynaecology and general practitioners attending a fertility workshop. (2) Twenty women (age 18-25) previously treated for cancer were offered a fertility consultation, AMH blood test and antral follicle count).

Results: (1) 94% of participants (97/103) desired more knowledge about FP options. 99% (102/103) either 'agreed' or 'strongly agreed' that patients would benefit from a clear referral pathway for FP. (2) 15 young women (75%) enrolled within one week of receiving the invitation to participate. To date, 10/15 (66%) have attended for assessment. 5/10 (50%) received treatment pre-menarche, 4/10 (40%) post-menarche and 1/10 had menarche during treatment. Menstrual cycles occurred following treatment in 100% (10/10). AMH levels ranged from 9-26.2 pmol/l; AFC ranged from 12-30. 4/10 said fertility was never discussed before treatment; 6/10 said fertility was just briefly mentioned. 9/10 (90%) were unaware of the AMH blood test. All participants were aware of "egg freezing" and would "probably" or "definitely" opt to freeze if told they had low ovarian reserve.

Conclusion: There is an acknowledged need for education of HCPs and a clear pathway of referral for FP in paediatric oncology in Ireland. Female survivors of childhood cancer have a strong desire for fertility assessment and FP if required. These findings highlight the need to develop a national paediatric FP service.

1.Algarroba GN, Sanfilippo JS, Valli-Pulaski H. Female fertility preservation in the pediatric and adolescent cancer patient population. Best practice & research Clinical obstetrics & gynaecology. 2018 Apr;48:147-57. PubMed PMID: 29221705.

P052 Emergency sperm and spermatogonial stem cell retrieval in oncological context

Micol Lionel A.¹; Williamson Elizabeth²; Sangster Philippa²

Background: Patients receiving chemotherapy or bone marrow transplant in oncological or haematological context are at high risk of losing their fertility hence the recommendation for sperm cryopreservation from semen. However, patients presenting with either azoospermia or inability to provide semen, face the risk to have to sacrifice their possibility to father their biological children. To those patients our andrology unit aims at offering timely preservation of their fertility by surgical sperm/stem cell retrieval.

Methods: Patients were enrolled prospectively and data analysed retrospectively.

Results: We screen on annual basis about half a thousand patients necessitating chemotherapy or bone marrow transplant out of which 10.5% present azoospermia. From January 2018 to July 2019, 24 patients (median age of 16.5 years old and average testosterone at 6.5 nmol/L) have chosen to go forward with surgical sperm/stem cell retrieval which could be offered within 5-day as a median from semen-analysis and preceding on average less than two days from chemotherapy start date. Primary diagnoses were leukaemia in 6 patients, sarcoma in 5 patients, testicular tumour in 5 patients, lymphoma in 4 patients, multiple myeloma in 1 patient and prostate cancer in 1 patient, while 2 patients had non-oncological haematological diseases. In terms of intervention 7 patients had standard testicular sperm extraction (TeSE), 5 had oncoTeSE, 3 patients had electroejaculation and TeSE, 1 had testicular sperm aspiration and 6 had testicular wedge biopsy for spermatogonial stem cell retrieval. The success rate for surgical sperm retrieval was 77.8% to which could be added the 5 patients who had spermatogonial stem cell retrieval. The average Johnsen score of testicular biopsies was 4.87 +/- 2.18.

Conclusion: We show that emergency sperm and spermatogonial stem cell retrieval in oncological context is a valid treatment option with high success rate for patients in which sperm cryopreservation from semen is impossible.

P053 Fertility preservation in female cancer patients post-chemotherapy treatment

Ward Hannah; Yasmin Ephia

University College London Hospital

Introduction: Advances in the field of oncology has led to increasing cancer survival rates (1). Many of these treatments are detrimental to womens' future reproductive potential, and following cytotoxic therapies, ovarian failure is common (2). Therefore, more women are opting to have fertility preservation prior to treatment. Reproductive preservation is increasing in importance, as an increasing number of young women survive cancer.

Methods: A cohort study was conducted, looking at 163 females of reproductive age, diagnosed with varying types of cancer, who underwent fertility preservation, at a large teaching hospital. Patients were separated according to primary malignancy and whether chemotherapy was received prior to egg retrieval. The number of eggs retrieved and anti-mullerian hormone (AMH) level were recorded in each patient.

Results: The average AMH level and number of eggs retrieved was higher in those who had not received chemotherapy prior to undergoing fertility preservation. Average age of the non-chemotherapy group was 32 compared with 27 in those who received chemotherapy. Average pretreatment AMH level in non-chemotherapy patients was 20.5, which would indicate a normal ovarian reserve. This was compared to average sub-optimal AMH of 14.3 in individuals who received chemotherapy prior to treatment. Average number of eggs retrieved was 9.8 in those who had received chemotherapy, compared to 13 in those who had not.

Conclusion: Reproductive preservation has become one of the key aspects in cancer management. Studies have found that ovarian aging occurs earlier in cancer patients (3) even prior to initiation of treatment. This data suggests that on average chemotherapy reduces the amount of eggs retrieved and IVF success rates. It is imperative that cancer patients of reproductive age are informed and offered fertility preservation for future IVF, prior to treatment if possible.

P054 Extracorporeal retrieval and vitrification of oocytes for fertility preservation

Pike Vanessa¹; Bird Sophie²

¹Beginnings ACU, St Helier Hospital; ²Beginnings ACU

Objective: Report a case of extracorporeal oocyte retrieval from unilateral oophorectomy in an ovarian cancer patient.

Patient: A 32 year-old single woman, gravida 0, with previous oophorectomized left ovary and endometrioid adenocarcinoma of the right ovary was scheduled for oophorectomy. Transvaginal egg collection was considered unsafe due to risk of malignant cell spillage and cancer upstaging.

Method(s): Ovarian stimulation of patient using flare protocol with 450IU of Menotrophin (FSH/LH) and letrozole. An ovulatory trigger was administered 34 hours prior to the scheduled oophorectomy. Oophorectomised tissue was transported to the IVF laboratory for ex-vivo follicular aspiration of oocytes. Mature oocytes vitrified 37.5 hours post trigger.

Result(s): Six oocytes retrieved, five mature and vitrified successfully. Oophorectomy successful. No ovarian cortex tissue cryopreserved, oophorectomized tissue sent to histology post-aspiration.

Conclusion(s): This case demonstrates that extracorporeal oocyte retrieval in patients with ovarian cancer can lead to successful fertility preservation without the risk of malignant cell spillage and cancer upstaging.

P055 Double ovarian stimulation (duostim) modified protocol for fertility preservation in female oncology patients - the way ahead! - our experience of 2 years in a tertiary care IVF unit

Sarodey Geet; Manukian Barbara; Balfoussia Dana; Saravelos Sotirios; Lavery Stuart; Salim Rehan

Wolfson Fertility Centre, IVF Hammersmith, London

Objective: Oncology patients have a window of period when we can optimise oocyte harvest. Our objective is to get the maximum oocytes in the minimum amount of time available. It is known that the Duostim approach with luteal phase stimulation after first egg collection is beneficial.

Methods: A retrospective analysis of luteal phase stimulation in patients for fertility preservation in oncology patients in a tertiary care IVF unit over a period of two years from 2017 - 2019. All patient records were retrieved from the IDEAS database and results evaluated in terms of ovarian response to second phase of luteal stimulation and the number of oocytes collected. The outcome was measured in terms of mature oocytes / embryos available to freeze. Our stimulation protocol is a modified Duostim method - rFSH used for stimulation with GnRH antagonist from day 5 of stimulation and trigger with GnRH agonist followed by the first oocyte collection. The luteal phase of stimulation started with same protocol after 4 days of egg retrieval consecutive second egg retrieval was done after GnRH agonist trigger. Observations: 15 patients for fertility preservation due for oncology treatment were analysed. Total number of oocytes retrieved 1st egg collection - 121(91 metaphase II), 2nd egg collection - 134 (111 Metaphase II). GnRh agonist trigger caused complete luteolysis, there was no increased bleeding at the second oocyte retrieval. No patients had Ovarian hyperstimulation. There was good patient satisfaction. No oncology treatments were delayed. Luteal phase collection yielded more oocytes.

Conclusions: Duostim protocol with GnRH Agonist trigger seems to be the way ahead to maximise the number of oocytes preserved for female oncology patients. This method has been popular with poor responders and provides a good option for fertility preservation patients. This is the first study with GnRH agonist trigger on oncology patients.

References: 1. Tsampras N, Gould D, Fitzgerald CT, et al. Double ovarian stimulation (DuoStim) protocol for fertility preservation in female oncology patients. *Hum Fertil (Camb)*. 2017 Dec;20(4):248-253. doi: 10.1080/14647273.2017.1287433. Epub 2017 Feb 9. 2. Ubaldi F, Capalbo A, Vaiarelli A, et al. Follicular versus luteal phase ovarian stimulation during the same menstrual cycle (DuoStim) in a reduced ovarian reserve population results in a similar euploid blastocyst formation rate: New insight in ovarian reserve exploitation. *Fertil Steril*. 2016;105(6):1488-95.

P056 Awareness level, knowledge and attitude toward fertility preservation among cancer patients in Makkah Region: A cross sectional study

Sindi Ramya

Umm Al-Qura University

Introduction: Fertility of cancer patients can be threatened by chemotherapy and gonadal irradiation during the course of cancer treatment^{1,2}. Little is known about fertility services and the role of health practitioner regarding fertility preservation options for cancer patients in Saudi Arabia. The aim of this study was to evaluate the awareness level, knowledge and attitude toward fertility preservation among cancer patients in Makkah region through a cross-sectional study.

Materials and Methods: In this cross-sectional study, 132 cancer patients from Makkah region aged 14 to 85 years old were asked to complete an approved closed-ended questionnaire contained 17 questions about patients' sociodemographic characteristics, knowledge and attitude towards fertility preservation. The data were analyzed using GraphPad Prism (version 8.0) software.

Results: The study revealed that about 50% of respondents were aware about fertility preservation. The majority of participants had never discussed fertility preservation options with their consultant/doctor nor referred to fertility clinic before commencing the treatment. Most patients agreed that the Saudi Ministry of Health should increase their awareness role toward fertility preservation and recommended public fertility services for cancer patients.

Conclusion: The current study showed good awareness level towards fertility preservation among cancer patients in Makkah region. However, knowledge and practice attitude among general public and clinicians remains insufficient. Government fertility services and referral center should set up to enhance general knowledge and attitude about available options for fertility preservation. Further studies in the field of cancer care and fertility preservation rights in Saudi Arabia are required.

1. AJALA, T., RAFI, J., LARSEN-DISNEY, P. & HOWELL, R. 2010. Fertility preservation for cancer patients: a review. *Obstet Gynecol Int*, 2010, 160386. 2. AL-AMOUDI, S. M. 2017. Health empowerment and health rights in Saudi Arabia. *Saudi medical journal*, 38, 785.

P057 Is there gender bias in the peer review system at Reproduction?

Biolkova Marie¹; Moore Tom²; Schindler Karen³; Swann Karl⁴; Fitzharris Greg⁵; Price Christopher⁵; Flook Lindsay⁶; Dick Helen⁶; Spears Norah¹

¹University of Edinburgh; ²University College Cork; ³Rutgers University; ⁴Cardiff University; ⁵University of Montreal; ⁶Bioscientifica

For researchers, success largely depends upon publication of their work in peer-reviewed journals. Recent evidence indicates that gender bias exists in the peer review process thereby negatively impacting careers. To determine if authors submitting to Reproduction are impacted by gender bias, we analysed submissions that underwent their peer review between 2007 and 2019. The gender of authors and reviewers was predicted from first names and their countries of affiliation, using the genderize.io tool. The requirements for using data included gender predictability with minimum 95% reliability, and at least 55% of a country's unique names passing that reliability threshold. This filtering resulted in around two thirds of data being usable for subsequent analyses. The relatively small number of review articles (n=390) made it difficult to reach firm conclusions. Analysis of research papers (n=4235) showed that first authors were more often female (57.8%), with last authors usually male (64.2%). No significant correlation was found between outcome and gender, for either first or last author. Subtle differences were found, though, at the editorial and

reviewer level. Manuscripts were more likely to be accepted where female Associate Editors (AEs) were handling papers with female last authors ($p < 0.05$). No bias was observed for male AEs and last authors ($p = 0.503$), but importantly overall there was no significant difference in the acceptance rates of female and male AEs for female and male last authors ($p = 0.138$). Female AEs were more likely to appoint female reviewers than were male AEs ($p < 0.001$). Reviewers were more likely to give better recommendations to research papers with female last authors than to those with male last authors ($p < 0.05$): when female and male reviewers were examined separately, this trend was significant for female reviewers only ($p < 0.05$). Overall the survey suggests a lack of any major gender-related bias in the peer review process at Reproduction.

P058 Top ten priorities for future infertility research

Duffy James M. N.¹; Wilkinson J²; Lensen S³; Vercoe M³; Bofill M³; Bhattacharya S⁴; Bhattacharya S⁴; Collura B⁵; Curtis C⁶; Evers J⁷; Farquharson RG⁸; Franik S⁹; Hickey M¹⁰; Hull L¹¹; Nagels H³; Johnson NP¹¹; Jordon V³; Khalaf Y¹²; Knynenburg J¹³; Legro RS¹⁴; Mol BW¹⁵; Sadler L³; Otter AS¹⁶; Mavrelos D¹⁷; Niederberger C¹⁸; Ng EHY¹⁹; Puscasiu L²⁰; Rautakallio-Hokkanen S²¹; Repping S²²; Stewart J²³; Strandell A²⁴; Strawbridge C²⁵; Showell M³; Torrance HL²⁶; Vail A²; van Wely M²²; Vuong NL²⁷; Wang AY²⁸; Wang R¹¹; Youssef MA²⁹; Farquhar CM³

¹Balliol College, University of Oxford; ²University of Manchester; ³University of Auckland; ⁴University of Aberdeen; ⁵RESOLVE: The National Infertility Association; ⁶University of Waikato; ⁷University Medical Centre Maastricht; ⁸Liverpool Women's NHS Foundation Trust; ⁹Münster University Hospital; ¹⁰University of Melbourne; ¹¹University of Adelaide; ¹²Kings College London; ¹³Freya Dutch Infertility Association; ¹⁴Penn State College of Medicine; ¹⁵Monash University; ¹⁶Osakidetza OSI; ¹⁷University College Hospitals; ¹⁸University of Illinois at Chicago College of Medicine; ¹⁹The University of Hong Kong; ²⁰University of Medicine and Pharmacy, Targu Mures; ²¹Fertility Europe; ²²Academic Medical Centre; ²³British Fertility Society; ²⁴Sahlgrenska University Hospital; ²⁵Fertility Network UK; ²⁶University Medical Centre Utrecht; ²⁷University of Medicine and Pharmacy at Ho Chi Minh City; ²⁸University of Technology Sydney; ²⁹Cairo University

Introduction: Despite the escalation in research activity and an exponential rise in published papers, many of the fundamental questions about the treatment of infertility remain. This is a barrier to improving the care people with infertility receive.

Objective: To identify and prioritise important research questions for infertility.

Methods: An international Priority Setting Partnership was established, including healthcare professionals, researchers, and people with infertility. Research uncertainties were gathered from healthcare professionals, researchers, and people with infertility. These research uncertainties were prioritised in a transparent process, using robust consensus science methods advocated by the James Lind Alliance (1).

Results: In the initial survey, 179 healthcare professionals, 28 researchers, and 153 people with infertility, submitted 423 research uncertainties. A review of clinical practice guidelines and Cochrane systematic reviews identified a further 236 research uncertainties. A long list of 231 research questions was entered into an interim prioritisation survey which was completed by 103 healthcare professionals, 28 researchers, and 169 people with infertility. Prioritised research uncertainties were entered into a consensus development conference. Using the modified Nominal Group Technique, 17 healthcare professionals, seven researchers, and 14 people with infertility prioritised the top ten research uncertainties for infertility.

Conclusions: We anticipate a prioritised list of research uncertainties, developed to specifically highlight the most pressing clinical needs as perceived by people with infertility and healthcare professionals working in the field, will help funding organisations and researchers to set their future research priorities. We are drowning in research that is singularly lacking in impact. Our approach should ensure future research has the necessary reach and relevance to inform future clinical practice and improve patient outcomes.

(1) James Lind Alliance. The James Lind Alliance Guidebook. Southampton, United Kingdom; 2018.

P059 Developing a minimum data set, known as a core outcome set, for future infertility research

Duffy JMN¹; Vercoe M²; Alahwany H³; Bhattacharya S⁴; Collura B⁵; Curtis C⁶; Evers JLH⁷; Farquharson RG⁸; Franik S⁹; Giudice L¹⁰; Khalaf Y¹¹; Knijnenburg JML¹²; Leevers B¹³; Legro RS¹⁴; Lensen S²; Martinez-Vazquez JM¹⁵; Mavrelou D¹⁶; Mol BW¹⁷; Niederberger C¹⁸; Ng EHY¹⁹; Otter AS²⁰; Puscasiu L²¹; Rautakallio-Hokkanen S²²; Repping S²³; Simpson JL²⁴; Strandell A²⁵; Strawbridge C²⁶; Torrance HL²⁷; Vail A²⁸; van Wely M²³; Vuong NL²⁹; Wang AY³⁰; Wang R³¹; Wilkinson J²⁸; Youssef MA³²; Farquhar CM²

¹Balliol College, University of Oxford; ²University of Auckland; ³University of Nottingham; ⁴Cardiff University; ⁵RESOLVE: The National Infertility Association; ⁶University of Waikato; ⁷Maastricht University Medical Centre; ⁸Liverpool Women's NHS Foundation Trust; ⁹Münster University Hospital; ¹⁰University of California; ¹¹Kings College London; ¹²Freya Dutch Infertility Association; ¹³University Hospital Zurich; ¹⁴Penn State College of Medicine; ¹⁵Cochrane Iberoamerica; ¹⁶University College Hospitals; ¹⁷Monash University; ¹⁸University of Illinois at Chicago College of Medicine; ¹⁹The University of Hong Kong; ²⁰Osakidetza OSI; ²¹University of Medicine, Pharmacy, Sciences and Technology, Targu Mures; ²²Fertility Europe; ²³Amsterdam University Medical Centres; ²⁴Florida International University; ²⁵University of Gothenburg; ²⁶Fertility Network UK; ²⁷University Medical Centre Utrecht; ²⁸University of Manchester; ²⁹University of Medicine and Pharmacy in Ho Chi Minh City; ³⁰University of Technology, Sydney; ³¹University of Adelaide; ³²Cairo University

Background: Complex issues, including a failure to consider the perspectives of people with fertility problems when selecting outcomes, variations in outcome definitions, and outcome reporting bias, make the results of infertility research difficult to interpret.

Objectives: To develop a core outcome set to standardise outcome selection, collection, and reporting across future randomized controlled trials and systematic reviews evaluating potential treatments for infertility.

Methods: Healthcare professionals, researchers, and people with fertility problems were brought together in an open and transparent process using formal consensus science methods including a three-round Delphi survey (372 participants from 41 countries) and consensus development workshop (30 participants from 27 countries) (1).

Results: The core outcome set consists of: (1) viable intrauterine pregnancy confirmed by ultrasound (accounting for singleton, twin, and higher multiple pregnancies); (2) pregnancy loss (accounting for ectopic pregnancy, miscarriage, stillbirth, and termination of pregnancy); (3) live birth; (4) gestational age at delivery; (5) birth weight; (6) neonatal mortality; and (7) major congenital anomaly. Time to pregnancy leading to live birth should be reported when applicable.

Conclusions: Embedding the core outcome set within randomized controlled trials and systematic reviews should ensure the comprehensive selection, collection, and reporting of core outcomes. Research funding bodies, the Standard Protocol Items: Recommendations for Interventional Trials (SPIRIT) statement, and over 80 specialty journals, including Cochrane Gynaecology and Fertility Group, Fertility and Sterility, and Human Reproduction, have committed to implementing this core outcome set.

(1) Duffy JMN, Bhattacharya S, Curtis C, Evers JLH, Farquharson RG, Franik S, et al. A protocol developing, disseminating and implementing a core outcome set for infertility, Human Reproduction Open. 2018;3:hoy007.

PO60 Criteria for IVF funding in the NHS are not fit for purpose

Platts Sophie¹; Mavrelou Dimitrios²

¹Univeristy College Hospital London; ²University College Hospital London

Background: The provision of in vitro fertilisation treatment (IVF) within the UK NHS varies across the country, and often depends on local Clinical care groups (CCG) policies. Many CCGs ration the provision of IVF to infertile couples based on ovarian reserve criteria (ORT). Different CCGs have different criteria which affords an opportunity to examine whether treatment eligibility criteria relating to ORT are fit for purpose.

Methods: Retrospective cohort study of couples undergoing IVF treatment between January 2014 and December 2016 at the Reproductive Medicine Unit (RMU). We excluded (i) cycles that involved gamete donation (ii) unstimulated cycles; (iii) cycles that did not result in any embryo transfer. Primary outcome was cumulative livebirth rate (CLBR), calculated as the number of women who had livebirth after starting a cycle of ovarian stimulation and transfer of all

resulting embryos over number of women starting a cycle of ovarian stimulation in the study period. Patients who had not had a livebirth but had frozen embryos left, discarded or transferred out were included and categorised as not having livebirth, giving a "pessimistic" estimate of CLBR.

Results: During the study period 445 couples underwent first cycle of ovarian stimulation. 97/445, 21.8% (95% CI 0.18-0.26) were classified as having low ORT as defined by one of the local CCGs. There was no difference in live birth rate after initial transfer between women with low and normal ORT [45/97, 46.4% (95% CI 0.37-0.56) vs. 143/348, 41.1% (95% CI 0.36-0.46)] ($p=0.35$). There was a significant difference in CLBR between women with low and normal ORT [53/97, 54.64% (95% CI 0.45-0.65) vs. 243/348, 69.83% (95% CI 0.65-0.75)] ($p=0.007$).

Conclusions: Restrictive criteria based on ORT set by CCGs to ration NHS IVF provision are resulting in couples losing the opportunity to have a biological family.

P061 Should mitochondrial replacement therapy be funded by the national health service?

Rhys-Evans Sophie; Frith Lucy

University of Liverpool

The first clinical trial on Mitochondrial Replacement Therapy (MRT) is currently being conducted and if this technique proves to be safe, MRT will be funded by NHS England through the Highly Specialised Services (HSS) funding stream. This paper will consider whether MRT should be publicly funded on the NHS. Given the current financial pressure the NHS is experiencing, it is essential that a comprehensive discussion is had with all viewpoints considered before it is commissioned by the HSS. There is yet to be a thorough discussion on MRT funding, perhaps because it is a small-scale issue and presumed to be covered by the HSS budget. However, the source of funding has not yet been confirmed due to the trial's incompleteness. This paper will consider whether MRT should be publicly funded on the NHS. If the trial concludes successfully, these funding decisions will have to be made, and therefore it is important to consider the arguments surrounding NHS funding of MRT in advance, so the discussion can be started, and robust and reasoned decisions made over the allocation of scarce NHS resources. The arguments both against and in favour of MRT being funded on the NHS will be evaluated. The argument against NHS funding is that the HSS is already overspending its budget in an underfunded NHS, suggesting funding needs to be re-prioritised. This discussion leads on to a comparison with in-vitro fertilisation (IVF), noting that funding for assisted conception services is already limited and therefore funding MRT could possibly restrict this further. Secondly, the ethical issue of allowing public access to a technique with such little evidence behind it will be explored. Finally, I will consider how privately funding MRT would affect the treatment's development. After evaluating all arguments, I will conclude that MRT should be funded by the NHS.

P062 Does the provision of information influence patients' decisions when selecting the number of embryos for transfer during an ART cycle?

Ang Melissa

Manchester University Foundation Trust

Background: The transfer of multiple embryos within a single assisted reproductive technology (ART) cycle increases the chance of a multiple pregnancy occurring and hence maternal and foetal complications. In Asia where ART is often sought from private providers, the costs involved often prompt patients to opt for multiple embryo transfers within single treatment cycles in hopes of achieving successful outcomes. The aim of this study was to determine if the provision of information on the risks of multiple pregnancy influences patient preference for double embryo transfer in years 2015 and 2018.

Methods: This project was conducted over two separate three-week periods in July 2015 and June 2018 in a private fertility clinic in Malaysia. An online self-administered questionnaire was utilised and patients were randomly selected to participate. The same questionnaire was completed twice by each patient, both before and after the provision of written information on the risks of multiple pregnancy. The information gathered include patient demographics,

pregnancy and fertility history, awareness about the risks of multiple pregnancy and their personal preference on the number of embryos transferred during their ART cycle.

Results: The results demonstrate no significant difference in patients' awareness about the risk of multiple pregnancy between years 2015 and 2018. However, 85.7% of participants in 2015 still opted for double embryo transfer after the provision of information, while this had reduced to 59.2% ($p < 0.05$) in 2018.

Conclusion: This study shows that in both years, patients are inclined to have double embryo transfers to achieve higher pregnancy rates despite knowing the risks involved. The provision of information is useful in influencing their decision on this matter but the extent of this influence needs to be further investigated.

P063 Awareness of age-related female fertility decline amongst medical students: A cross-sectional study

Zia Nawal¹; Pattani Nikhil¹; Cheshire James²; Chu Justin³

¹University of Birmingham; ²Institute of Metabolism and System Research; ³Birmingham Women's and Children's NHS Foundation Trust

Background: With women now tending to delay parenthood, there are marked implications for IVF treatment success rates, given the factor of age-related fertility decline. It is imperative that couples are aware of this before making decisions, yet current PSHE guidelines for secondary schools place no emphasis on fertility education.

Objectives: This study aimed to establish the current standard of knowledge amongst medical students and proposes how we can improve this in secondary schools around the UK.

Methods: 152 consenting medical students completed a questionnaire which asked about exposure to fertility education during PHSE classes.

Results: Only 20/152 (13.2%) were educated on fertility education; yet 139/152 (92%) thought that it is an important aspect of the PSHE curriculum. When asked at what age does a woman's fertility start to decline, answers ranged between 18 to 60 years old.

Conclusions: There is a clear gap in knowledge, which calls for a need to implement fertility education into the PSHE curriculum. The consequences of this action can be vast and wholly beneficial, leading to more informed choices, and less economical and emotional burden on future mothers-to-be.

P064 Complications encountered by patients post IVF/ICSI at regional tertiary referral NHS fertility unit over a two year period

McManus John Philip¹; Agnew Heather¹; Bailie Emily²; Campbell Nicola³; McMullan Courtney⁴; Snowdon Adam⁵; Agbaje Ishola¹

¹Belfast Health and Social Care Trust; ²Northern Health and Social Care Trust; ³Southern Health and Social Care Trust; ⁴South Eastern Health and Social Care Trust; ⁵Western Health and Social Care Trust

Objectives: Although assisted conception is very safe, some patients encounter complications requiring hospital admission. This study reviews the management and outcomes in the two types of admission post IVF/ICSI over a 2 year period at a regional NHS fertility centre: ovarian hyperstimulation (OHSS) and non-OHSS complications.

Methods: Patients were screened for admissions post treatment during 2016-2017. Whilst NHS IVF/ICSI treatments occur at the regional centre, patients are often admitted to their local hospital if complications arise. Therefore, data collection was at a regional level with collaboration from all relevant healthcare trusts. Medical records and regional information systems were interrogated for data.

Results: 1626 cycles of IVF/ICSI took place. Forty four (2.7%) patients were admitted with OHSS: 16 (1%) severe, 14

(0.9%) moderate and 14 (0.9%) mild.

Ten (0.6%) were admitted for at least seven days. 6/44 (13.6%) had all initial investigations according to regional guidelines. 30/44 (68.5%) had appropriate inpatient monitoring. All patients received appropriate VTE prophylaxis. Four patients (0.2%) required chest drainage. Four patients (0.2%) required paracentesis. 14.3% of successful embryo transfers did not receive first trimester enoxaparin.

26 (1.6%) patients were admitted for non-OHSS complications relating to treatment; reasons included:

- 3 (0.2%) pelvic abscesses
- 3 (0.2%) cyst related
- 2 (0.1%) surgically managed ectopic pregnancies
- 5 (0.3%) infection
- 8 (0.5%) ovarian torsion/intermittent torsion

Eight (0.5%) required surgery for complications relating to treatment. Thirteen (0.8%) patients were admitted for >2 days and/or underwent surgery.

There were no ICU admissions.

Conclusion: The number of admissions are small, equating to 70 patients (4.3% of cycles). Of these, 29 (1.8%) had a serious complication (severe OHSS or complicated non-OHSS admission). A significant number of OHSS related admissions did not have appropriate investigations or monitoring. Improvements are required in regional reporting, communication and guidance in the management of complications

P065 'Group culture': Embryology perspectives of working within a large cohesive group of IVF clinics

Campbell Alison; Smith Rachel; Best Louise; Montgomery Sue; Berrisford Kathryn; Nice Lynne; Armstrong Ellen; Lodge Yvonne; Page Alex; Corcoran Sharon; Drezet Cath

CARE Fertility Group

This abstract aims to discuss the pros and cons of 'group culture' for the embryologist and the benefits and challenges of a multi-centre, aligned best practice strategy. Many IVF clinic groups exist internationally, with reportedly varying degrees of embryology standardisation. A cohesive approach brings advantages for the embryologist, the patient and the business. A large, diverse team of embryologists can combine experiences and identify synergies for continuously improving and aligned embryology practice. A structure with inter-clinic specialist teams can drive practice forward and offer embryologists opportunities to contribute to group-wide SOP development. Centrally managed, standardised documentation and procurement can reduce administrative, financial and operational burden. Inter-laboratory training, support, cross-cover, trouble-shooting, audit and mock inspection assure high standards with rapid detection and response to trends. Bench-marking of intra and inter-lab team results facilitates continuous improvement, providing a culture of transparency, healthy competition and progression. Large combined data enables reliable and rapid analysis of KPI, research, development, change control and validation. However, achieving alignment, avoiding drift in practice and change management is challenging and requires buy-in to this culture, and visibility of the benefits. Some local variation to practice may be necessary due to clinic premises or operational restrictions and effective information dissemination and communication is complicated by geographical spread. In conclusion, Acceptance of, and integration to 'group culture' can bring challenges on many levels. These drawbacks are considered to be outweighed by the benefits and economies of scale. An efficient, responsive and progressive group-wide embryology team with common practice, objectives and standards can achieve excellent and consistent outcomes for patients, whilst bringing demonstrable efficiencies for the clinics. This approach should direct positive change and continuous improvement at a pace which may be less attainable in a stand alone facility.

P066 Probiotic therapy in couples with infertility: a systematic review

Corbett Gillian; Crosby David; McAuliffe Fionnuala

Background: The reproductive microbiome is becoming increasingly recognised for its influence on fertility(1,2). While there is evidence to support the treatment of bacterial vaginosis (3,4) and disordered microbiomes(5) to optimise outcomes in Assisted Reproductive Technology (ART), the role of probiotics is yet to be established. The therapeutic potential of probiotic therapy remains an exciting opportunity in ART and this review endeavours to summarise its evidence to date.

Methods: A systematic review of all databases was performed to examine the evidence on probiotic therapy in couples with infertility undergoing on ART. The primary outcome was improvement in clinical pregnancy rate with probiotic therapy in ART. Secondary outcomes included improvement in male and female fertility parameters.

Results: The initial search found 882 articles, of which 26 full manuscripts were reviewed. Four articles were eligible for inclusion. Three of the four studies were of low quality. Two studies looked at vaginal probiotic therapy in women (6,7). Neither showed change in clinical pregnancy rate. Two studies looked at oral probiotic therapy in men (8,9). Neither of these studies included clinical pregnancy rate as an outcome but both showed some improved sperm motility.

Conclusion: There is limited high quality evidence examining the use of probiotic therapy in assisted reproduction. While some benefit in sperm motility has been observed with male probiotic therapy, no change in clinical pregnancy rate has yet been described. High quality randomized studies are needed to definitively examine probiotic therapy and establish its benefit for couples undergoing assisted reproduction.

1. Moreno I, Fransiak JM. Endometrial microbiota-new player in town. *Fertil Steril*. 2017;108(1):32-9. 2. Fransiak JM, Scott RT. Reproductive tract microbiome in assisted reproductive technologies. *Fertil Steril*. 2015;104(6):1364-71. 3. Chenoll E, Moreno I, Sánchez M, García-Grau I, Silva Á, González-Monfort M, et al. Selection of New Probiotics for Endometrial Health. *Front Cell Infect Microbiol*. 2019;9:114. 4. Larsson PG, Stray-Pedersen B, Rytting KR, Larsen S. Human lactobacilli as supplementation of clindamycin to patients with bacterial vaginosis reduce the recurrence rate; a 6-month, double-blind, randomized, placebo-controlled study. *BMC Womens Health*. 2008;8:3. 5. Russo R, Edu A, De Seta F. Study on the effects of an oral lactobacilli and lactoferrin complex in women with intermediate vaginal microbiota. *Arch Gynecol Obstet*. 2018;298(1):139-45. 6. Gilboa Y, Bar-Hava I, Fisch B, Ashkenazi J, Voliovitch I, Borkowski T, et al. Does intravaginal probiotic supplementation increase the pregnancy rate in IVF-embryo transfer cycles? *Reprod Biomed Online*. 2005;11(1):71-5. 7. Kyono K, Hashimoto T, Kikuchi S, Nagai Y, Sakuraba Y. A pilot study and case reports on endometrial microbiota and pregnancy outcome: An analysis using 16S rRNA gene sequencing among IVF patients, and trial therapeutic intervention for dysbiotic endometrium. *Reprod Med Biol*. 2019;18(1):72-82. 8. Valcarce DG, Genovés S, Riesco MF, Martorell P, Herráez MP, Ramón D, et al. Probiotic administration improves sperm quality in asthenozoospermic human donors. *Benef Microbes*. 2017;8(2):193-206. 9. Maretti C, Cavallini G. The association of a probiotic with a prebiotic (Flortec, Bracco) to improve the quality/quantity of spermatozoa in infertile patients with idiopathic oligoasthenoteratospermia: a pilot study. *Andrology*. 2017 May;5(3):439-444

P067 'Add-on' or take away: managing women with recurrent implantation failure with intralipids during assisted reproductive cycles

Manning Rashmi¹; Irving Laura²; Ebuomwan Isaac²

¹Royal Victoria Infirmary, Newcastle; ²Queen Elizabeth Hospital Gateshead

Background: In recurrent implantation failure (RIF) multiple embryos are transferred over at least two assisted reproductive cycles (ARCs), without implantation occurring. The definition however varies. There is limited evidence for managing RIF; a possible aetiology is increased natural killer cell activity, with Intralipids modulating this and improving reproductive outcomes, however data is conflicting (1, 2).

Objective: To examine clinical pregnancy rates (CPR) and live birth rates (LBR) of women receiving Intralipids during ARCs.

Methods: Retrospective case-note review to assess our unit's practice between December 2013-October 2017. Data reviewed included: Demographics, infertility aetiology, previous ARCs, current ARCs, additional intervention(s) and pregnancy outcomes. Descriptive statistics were used.

Results: 35 patients underwent 55 ARCs involving Intralipids. 63.6% of ARCs (35/55) involved pre-cycle endometrial scratch (ES). Overall CPR=34.5% (19/55) and LBR=20% (11/55); no patient experienced side effects/adverse events. Embryo quality in previous cycles: 67.3% (37/55) cycles met RIF criteria of having at least 4 good quality embryos transferred over 3 cycles; 87.3% (48/55) cycles met local RIF criteria of having at least 2 blastocysts transferred over 2 cycles. Previous pregnancy outcome: Patients who had never been pregnant previously had CPR=50% (10/20) and

LBR=25% (5/20) while patients who had been pregnant previously had CPR=25.7% (9/35) and LBR=17.1% (6/35). Subgroup analysis of ARCs with Intralipids plus ES compared with ARCs with ES alone: ARCs with both interventions had CPR=37.1% (13/35) and LBR=28.6% (10/35) while ARCs with ES alone (separate audit) had CPR=27.9% (17/61) and LBR=26.2% (16/61).

Conclusions: It initially appears that management of RIF with Intralipids was of some benefit with an overall LBR of 20%, however a LBR of 26.2% in a cohort of patients who had ES alone makes this unlikely. Addition of Intralipids during ARCs therefore has not improved pregnancy outcomes in our RIF patients, consistent with previous literature showing minimal benefit (3).

1) Coulam CB, Acacio B. Does immunotherapy for treatment of reproductive failure enhance live births? *Am J Reprod Immunol* 2012;67:296-304. 2) Dakhly DM, Bayoumi YA, Sharkawy M, Gad Allah SH, Hassan MA, Gouda HM, et al. Intralipid supplementation in women with recurrent spontaneous abortion and elevated levels of natural killer cells. *Int J Gynaecol Obstet* 2016; 135: 324-7. 3) Martini AE, Jasulaitis S, Fogg LF, Uhler ML, Hirshfeld-Cytron JE. Evaluating the Utility of Intralipid Infusion to Improve Live Birth Rates in Patients with Recurrent Pregnancy Loss or Recurrent Implantation Failure. *J Hum Reprod Sci.* 2018; 11(3): 261-268.

P068 Personalised stimulation for expected high responders during in-vitro fertilisation

Papathanasiou Thanos; Pullan Lucy; Protsyshyn Marianna; Riccio Teresa; Mawal Nausheen

Bourn Hall Clinic

Background: Women with above-average ovarian reserve may particularly benefit from individualization of the stimulation regimen, aiming to avoid excessive ovarian response leading to ovarian hyperstimulation syndrome(1). The aim of the study was to identify personalised stimulation regimens that optimise ovarian response for these women.

Methods: A cohort of 1058 consecutive first fresh cycles of women with Anti-Mullerian Hormone levels $\geq 25\text{pmol/L}$ (between January 2014 and March 2018) was retrospectively analysed.

Women received between 100iu-150iu rFSH stimulation in the context of either a downregulation or an antagonist protocol. A generalized linear model predicting ovarian response (number of retrieved oocytes) according to patient characteristics and drug regimen (dose, protocol) was constructed. This model provided the most satisfactory fit for the positively skewed 'oocyte' outcome(2).

Drug regimens for given patient groups were identified as 'optimal' if they were predicted to achieve a mean yield of 12 oocytes (the average of 10-14 oocytes). An equivalence margin of ± 1.5 oocytes was applied. To demonstrate equivalence, the 90% confidence intervals would have to 'fall' within the 12 ± 1.5 margin (two one-sided test methodology)(3).

Results: For normo-weight women, 100iu-112.5iu rFSH combined with an antagonist protocol is the approach most likely to achieve an optimal oocyte yield (AMH 25-37pmol/L: 11.7 oocytes 90%CI 10.6-12.8 with 100iu rFSH, 12.1 oocytes 90%CI 11.2-13.1 with 112.5iu rFSH). Overweight women benefit from higher stimulation doses and/or use of the agonist protocol (AMH 25-37pmol/L: 11.7 oocytes 90%CI 10.7-12.8 with 150iu rFSH on an agonist protocol, AMH $\geq 38\text{pmol/L}$: 12.1 oocytes 90%CI 11-13.3 with 150iu rFSH on an antagonist protocol, 12.1-12.5 oocytes with 100iu-112.5iu rFSH on an agonist protocol).

Conclusions: In expected high responders, use of a fixed stimulation dose does not appear to be an optimal strategy. Instead, we argue that the stimulation dose should be individualized in accordance with the choice of protocol (agonist or antagonist).

1. Lensen SF, Wilkinson J, Leijdekkers JA, La Marca A, Mol BWJ, Marjoribanks J, et al. Individualised gonadotropin dose selection using markers of ovarian reserve for women undergoing in vitro fertilisation plus intracytoplasmic sperm injection (IVF/ICSI). *Cochrane Database Syst Rev* [Internet]. 2018 Feb 1 [cited 2018 Mar 28];2:CD012693.

2. Ng VKY, Cribbie RA. Using the Gamma Generalized Linear Model for Modeling Continuous, Skewed and Heteroscedastic Outcomes in Psychology. *Curr Psychol* [Internet]. 2017 Jun 9 [cited 2019 May 23];36(2):225—35.

3. Lakens D. Equivalence Tests: A Practical Primer for t Tests, Correlations, and Meta-Analyses. *Soc Psychol Personal Sci* [Internet]. 2017 May [cited 2019 May 24];8(4):355—62.

P069 Ethnic variation in the risk of preterm birth following frozen embryo transfer: results from analysis of the HFEA database

Mascarenhas Mariano¹; Sharpe Abigail²; Balen Adam¹

¹Leeds Fertility; ²Leeds Teaching Hospitals Trust

Background: Women of minority ethnicity appear to have an increased incidence of preterm birth (PTB) following a fresh embryo transfer. It has been unclear as to whether the same holds good for frozen embryo transfers. We aimed to assess this using data provided by the Human Fertilisation and Embryology Authority (HFEA).

Methods: We assessed the outcome of 11 334 singleton live births following frozen embryo transfer between 2000 to 2016. This included frozen embryo transfers for women of White British (n=43 735), White Irish (n = 1 090), Indian (n=3 034), Bangladeshi (n = 319), Pakistani (n = 1946), Chinese (n = 520), Black African (n = 1400) and Black Caribbean (n = 277). We assessed the PTB rate (birth <37 weeks gestation) and early PTB rate (<32 weeks gestation). Adjustment was performed for confounding variables including patient age group, previous live births, number of previous embryo transfers, ovulatory disorder, male factor, tubal factor, endometriosis and number of embryos transferred.

Results: The PTB rate when compared with White British women was significantly higher for women of White Irish (12.3% vs 8.4%, aOR 1.553, 95% CI 1.014 to 2.380), Indian (11.1% vs 8.4%, aOR 1.378, 95% CI 1.061 to 1.791) and Pakistani (11.8% vs 8.4%, aOR 1.488, 95% CI 1.088 to 2.035) ethnicities. The early PTB rate was increased for White Irish (3.9% vs 1.2%, aOR 3.490, 95% CI 1.671 to 7.293), Indian (2.4% vs 1.2%, aOR 1.933, 95% CI 1.118 to 3.342), Pakistani (2.5% vs 1.2%, 95% CI 1.068 to 4.026) and Black African women (4.2% vs 1.2%, aOR 3.472, 95% CI 1.837 to 6.562).

Conclusions: There appears to be a statistically significant impact of ethnicity on risk of PTB following frozen embryo transfers, indicating a need for further research investigating the possible mechanisms behind this increased risk of adverse outcome.

P070 Prescription medication use patterns amongst men and women just prior to an IVF cycle: a retrospective cohort study

Rostand Eddie¹; Mascarenhas Mariano²; Sharpe Abigail³; Ibrahim Suhair³; Bhandari Harish²

¹University of Leeds; ²Leeds Fertility Clinic; ³Leeds Teaching Hospitals Trust

Background: Prescription medication use may impact fertility treatment and pregnancy outcomes, but information is scarce on prescription medication use patterns among women having IVF, and scarcer for their male partners.

Methods: Men and women having IVF were asked to complete a form detailing their current prescription medication use which was cross-checked by the nurse specialist. 400 case records of couples having IVF from 10/2016 to 11/2017 with their own gametes were retrospectively analysed. We excluded cycles undergoing pre-implantation genetic testing and any duplicate records from repeat cycles for the same couple. The medications used were also subdivided into indications for use and Food and Drug Administration (FDA) categories (1). Since the sample size was insufficient for reliable statistical analysis, a descriptive report of the prescription medication utilisation patterns has been provided.

Results: Prescription medications were used by 22.5% (90/400) women. Live birth rates were 32.2% (29/90) and 32.9% (102/310) for women who were on and not on prescription medications respectively. Asthma medications (n=22), levothyroxine (n=12), selective serotonin re-uptake inhibitors (SSRIs) (n=10), ferrous sulphate (n=8), and drugs for diabetes mellitus (n=7) were most frequently used. One medication (n=12 women) was FDA category A, 20 medications (n=29 women) were category B, 25 medications (n=62 women) were category C, six medications (n=6 women) were category D and one medication (n=1 woman) was category X. Prescription medications were used by 22% (88/400) men. The live birth rate for those on medication was 30.7% (27/88) compared to those not on medication 33.0% (103/312).

Conclusions: The study found that a large number of women are prescribed category C, D or X drugs when attempting ART, with an unclear effect on the success of ART.

References 1. FDA Categories [Internet]. [cited 2019 Apr 23]. Available from: <https://www.govinfo.gov/content/pkg/FR-2008-05-29/pdf/E8-11806.pdf>

P071 Does sperm DNA fragmentation affect the outcome in donor- recipient cycle using intracytoplasmic sperm injection (ICSI) for fertilization?

Rao Anamika; Ruddick Lydia

Manchester Fertility

Background: Male factor is the leading cause of infertility affecting up to 50% of the infertile couple. Sperm DNA damage has been identified as an important contributor to male infertility as well as poor outcomes following assisted reproduction treatment.

Objective: To identify the effects of different levels of sperm DNA fragmentation in oocyte donation Cycles using ICSI for fertilisation.

Method: Retrospective study of 63 donor- recipient cycle. All oocyte donors fulfilled HFEA criteria with regard to age and screening. All donors were stimulated with an antagonist protocol. Minimum of 6 mature eggs were allocated to the recipients. The procedure for endometrial preparation for oocyte recipients involved the use of estrogen and progesterone. Comet test was used to identify the sperm DNA fragmentation. Fresh oocytes from the egg donors were fertilized with their partner's sperms using ICSI. We evaluated the results associated with low, moderate and high sperm DNA fragmentation rate. χ^2 test was used to compare the difference between 2 groups.

Results: Our study showed no significant difference in the age of the women treated, the age of the partners, fertilization rates ($p=0.8$), utilization rate, implantation rate ($p=0.09$), miscarriage rate and live birth rate ($p=0.7$) with different levels of DNA fragmentation rate.

Conclusion: The observations from our study support the findings of studies done to identify the effects of DNA fragmentation in treatments with donor oocytes. Donor oocytes, as generally obtained from young and healthy women, may be better able to repair DNA damage in the sperm.

1) Kathryn C. Humm. Effects of increased male age in IVF and egg donation: is sperm DNA fragmentation responsible? Fertil Steril. 2013 Jan;99(1):30-6. 2) Hee-Jun Chi. significantly improved the pregnancy rate of patients with a high sperm DNA fragmentation index Clin Exp Reprod Med. 2017 Sep; 44(3): 132-140. 3) Simon et al. DNA damage has a negative association with Live birth rates after IVF. Reproductive Biomedicine online (2013) 26, 68-78L 4) Gat . DNA fragmentation index does not correlate with blastocyst euploidy rate in egg donor cycles. Gynecol Endocrinol 2018 Mar; 34(3) 212-216 5) Antonouli S. Impact of sperm DNA fragmentation on ICSI outcome in cases of donated oocytes. Arch Gynecol Obstet 2019 Jul; 300(1) 207-215

P072 Ethnicity affects the live birth rate following frozen embryo transfer: analysis of 64,530 frozen embryo transfer cycles from the HFEA database

Mascarenhas Mariano; Sharpe Abigail; Balen Adam

Leeds Fertility

Background: Ethnicity of the woman appears to influence the live birth rate following a fresh embryo transfer. Published literature appears to suggest that ethnicity does not impact live birth rates following frozen embryo transfers. We requested the Human Fertilisation and Embryology Authority (HFEA) for access to their data to provide a definitive answer to this question.

Methods: We analysed 1 048 576 cycles from the HFEA database from 1991 to 2016 and after excluding cycles irrelevant to our analysis (such as fresh cycles, donated gametes, unavailability of ethnicity data), 64 530 frozen embryo transfer cycles (including frozen embryo transfers for 43 735 White British, 1 090 White Irish, 3 034 Indian, 1 946 Pakistani, 1 400 Black African, 319 Bangladeshi, 520 Chinese and 277 Black Caribbean women) were analysed. Adjustment was performed for confounding variables including patient age group, previous live births, number of

previous embryo transfers, ovulatory disorder, male factor, tubal factor, endometriosis, number of embryos transferred and decade of treatment.

Results: The live birth rate per embryo transfer when compared with White British women was significantly lower for women of White Irish (23.4% vs 26.1%, aOR 0.854, 95% CI 0.738 to 0.989), Indian (25.2% vs 26.1%, aOR 0.947, 95% CI 0.906 to 0.989), Bangladeshi (21.1% vs 26.1%, aOR 0.865, 95% CI 0.787 to 0.950) and Pakistani (25.7% vs 26.1%, aOR 0.967, 95% CI 0.942 to 0.993) ethnicities.

Conclusions: Ethnicity appears to affect live birth rate following frozen embryo transfer refuting existing literature on the topic. The effect size is narrow reflecting the power of this study indicating the need for large scale registry data to power studies looking at perinatal outcomes.

P073 Clinical outcomes of 1086 consecutive egg donation cycles from a single UK centre; a comparison of fresh and frozen eggs

Linara-Demakakou Elena; Wang JinJun; Shah Trina; Gibson Grace; Nair Shailaja; Rahmati Mona; Macklon Nick; Ahuja Kamal

The London Women's Clinic

Background/ Purpose/Objectives: The demand for egg donation in the UK remains high and with improvements in egg vitrification it is now feasible for women to consider using frozen donor eggs. While studies have suggested slightly higher risks for obstetric and neonatal complications with donor eggs, the increasing use of eSET and comprehensive donor health check-ups should reduce such risks. This retrospective study examines clinical outcomes from fresh versus frozen eggs obtained from the UK's largest egg bank, The London Egg Bank (LEB).

Methods: An analysis for 1086 consecutive cycles of fresh and frozen donor egg recipients who had treatment at The London Women's Clinic, Harley Street for the period between 2014-2018 was performed. All oocytes were obtained from donors recruited by the LEB and those frozen were vitrified in our embryology laboratory in Harley Street. The main outcome measures included thaw survival rates, implantation, clinical pregnancy and live birth rates. In addition, the relative incidence of self-reported perinatal outcomes including small for gestational age and premature birth were assessed.

Results: Between 2014-2018, 602 frozen (534 patients) and 484 fresh egg donation cycles (358 patients) were carried out. The thaw survival rate from 4765 eggs was 94%. The average number of eggs available per patient was 8 for the frozen and 11 for the fresh group. Respectively, 1.3 and 1.4 embryos transferred per patient. There was no difference in implantation (38% vs. 44.5%, $p=0.06$), clinical pregnancy (46% vs 50%, $p=0.3$) or live birth rates (LBR) (37% vs 43%, $p=0.08$). Moreover, no difference in perinatal health outcomes was observed between the two groups.

Conclusion: These data indicate good outcomes for patients who prefer to receive donor IVF treatment within the UK and that these are similar whether frozen or fresh donated eggs are used.

P074 Comparison of treatment outcomes between frozen-thawed day 5 and 6 blastocyst transfers

Cheung Candice; Kasraie Jason; Sizer Andrew

Shrewsbury and Telford Hospital NHS Trust

Background: Previous studies demonstrated lower pregnancy rates associated with delayed blastulation in fresh cycles. However, the effect of slower developing blastocysts in frozen-thawed cycle is less clear. Whilst some studies reported lower pregnancy rates with day 6 (D6) blastocysts compared to day 5 (D5), others reported similar outcomes. We aim to compare the treatment outcomes of patients in our unit who undergone D5 frozen-thawed blastocyst transfers (FBT) to those who had D6 FBT.

Method: We conducted a retrospective cohort study in a single UK assisted conception unit. A total of 294 cycles of D5 FBT and 158 cycles of D6 FBT from January 2013 to December 2018 were included. All blastocysts were cryopreserved by vitrification. Oestradiol valerate was used to induce endometrial proliferation. All patients had a day 11 scan and where the endometrium thickness is >8mm, they commenced on 400mg cyclogest pessary twice daily. Blastocyst transfer is performed after 5 days of progesterone supplementation. A pregnancy test was performed 12 days after the transfer and if positive, an ultrasound scan was performed 3 weeks after the test.

Results: The mean age at freeze (32.0 vs 32.3), BMI (24.9 vs 24.8), previous number of treatment cycles did not differ between the two groups. The average number of blastocysts transferred were again similar (1.8 in the D5 vs 1.7 in D6). Although D5 blastocysts resulted in a higher biochemical pregnancy rate of 52.7% compared to 44.3% in the D6 group ($p < 0.05$), the clinical pregnancy rates (24.1% in D5 vs 28.5% in D6, $p = 0.15$) and livebirth rates (22.1% in D5 vs 24.1% in D6, $p = 0.31$) are comparable. There is no significant difference in the multiple birth rates (4.4% in D5 vs 3.2% in D6, $p = 0.26$).

Conclusion: D5 and D6 frozen-thawed blastocysts demonstrate similar clinical pregnancy and livebirth rates.

P075 Frozen thawed embryo transfer cycles: Clinical outcomes - our experience

Balakumar Vidhya; Milne Philip; Ramalingam Mythili; Kini Suresh

NHS Tayside

Objective: To evaluate the impact of clinical and embryological factors on the pregnancy outcomes of frozen thawed embryo transfer cycles.

Methods: Retrospective data on frozen thawed embryo transfer cycles was collected between January 2018 to July 2019, for a period of eighteen months. This was performed using the local computer database and review of case notes. Various parameters including woman's age, parity, body mass index (BMI), quality of embryo and pregnancy outcomes were analysed.

Results: During the study period, 398 women underwent a total of 427 cycles. Ninety six percent of these cycles were hormone replacement frozen embryo transfers (HR FET) and 4 % were natural cycles. The average age of the woman was 35yrs and the mean BMI was 24. A total of 451 embryos were transferred with a 98.5 % post thaw survival rate. Majority of the transferred embryos (74%) were of Day 5. Among these, expanded blastocysts were used in 56% of the cycles followed by both blastocyst and hatching blastocyst transfers in 22% cycles each. The overall pregnancy rate was 55%, of which the clinical pregnancy rate of 70.9%, miscarriage rate of 28.7% and ectopic rate is 0.4% in the HR FET cycles.

Conclusion: Although various studies suggest that both natural cycle FET and HR FET protocols are equally effective in terms of pregnancy outcomes in women with regular menstrual cycles, majority of our cycles are HR FET cycles with good clinical pregnancy outcomes. Following a recent large multicentre cohort study (French, 14421 cycles) suggesting HR FET is associated with increased miscarriage rates compared to natural cycles, we are advocating to increase our natural cycles in our unit to see whether we can reduce our miscarriage rates. Re-audit will be conducted to see the effectiveness.

P076 Comparison of Treatment Outcomes between Fresh Day 5 and 6 Blastocyst Transfers

Cheung Candice; Kasraie Jason; Sizer Andrew

Shrewsbury and Telford Hospital NHS Trust

Background: The majority of embryos develop into blastocysts day 5 (D5) post egg collection; however, some embryos develop more slowly becoming blastocysts on day 6 (D6). Previous studies have suggested higher aneuploidy rates, reduced viability and lower implantation potential due to embryo-endometrial asynchrony on D6. We therefore

compared the treatment outcomes of patients in our unit who had blastocyst transfers on D5 to those with delayed blastulation and had transfer on D6.

Method: We conducted a retrospective cohort study in a single UK assisted conception unit. The treatment outcomes of 481 D5 blastocyst transfer cycles and 143 D6 transfers from January 2013 to December 2018 were analysed. All patients included fulfilled our elective single embryo transfer criteria and had single blastocyst transfer in a fresh autologous cycle. Embryos were assessed on day 5 post egg collection, if no blastocysts are graded 3BB or above, they were further cultured to D6 and transferred in the same cycle, any surplus embryos were vitrified for potential use in the future.

Results: The mean age (31.5 vs 31.6) and BMI (24.7 in both groups) did not differ between the groups. The D5 group had a higher mean number of oocytes collected (15.8 vs 14.0, $p < 0.01$) and fertilised (11.0 vs 8.9, $p < 0.01$) with a higher number of blastocysts available for vitrification (4.7 vs 1.7, $p < 0.01$) compared to the D6 group. In terms of treatment outcome, D5 transfer resulted in a higher biochemical pregnancy rate (58% vs 49%, $p < 0.03$), clinical pregnancy rate (49% vs 38%, $P = 0.01$) and livebirth rate (44% vs 33%, $p = 0.01$). There is no difference in monozygotic twinning rates (1.3% vs 1.4%, $p = 0.45$).

Conclusion: Although D5 blastocyst transfer demonstrates higher pregnancy and livebirth rates, D6 blastocyst still provides a reasonable chance in achieving a livebirth.

P077 Timing of embryo transfer in natural cycle frozen-thawed embryo replacement. Is blastocyst transfer on day six after urinary luteinising hormone-surge superior to day seven?

Noble Matt; Craig Jo; Bevan Aysha; Child Tim

Oxford Fertility

Background: Between 2012 and 2017, the annual number of frozen-thawed embryo replacement (FER) cycles in the United Kingdom doubled, while fresh cycles declined. Natural cycle FER (NC-FER) offers the advantage of avoiding medication. However, there is debate regarding embryo transfer timing. Aim: We asked, "Is it better to transfer a frozen-thawed blastocyst on the sixth or seventh day after luteinising hormone (LH) surge is detected on urine testing?"

Methods: We retrospectively analysed clinical outcomes following a change in protocol in May 2018 from blastocyst transfer on the seventh day after urinary LH surge (LH+7) to the sixth day (LH+6). All patients undergoing NC-FER of vitrified-thawed, unbiopsied blastocyst(s) derived from autologous oocytes between January 2017 and March 2019 were included. Outcomes were compared using logistic regression. The primary outcome was ongoing pregnancy (>24 weeks). Other aspects of practice remained unchanged and, as an independent control, medicated FER outcomes were monitored.

Results: 561 cycles were included (180 LH+6; 381 LH+7). Baseline characteristics were similar, including age at FER and oocyte recovery, endometrial thickness, number of embryos transferred and embryo quality. Ongoing pregnancy rate (OPR) was significantly higher when blastocyst transfer occurred on LH+6 compared to LH+7 (45.0% v 29.1%, $p < 0.0001$). Adjusting for age at oocyte recovery, endometrial thickness and number of embryos transferred the odds ratio was 2.15 (95% CI: 1.47-3.13, $p < 0.0001$). Biochemical pregnancy rate (61.7% v 32.8%) and clinical pregnancy rate (52.2% v 32.8%) were significantly ($p < 0.0001$) higher in the LH+6 group. Miscarriage rate was similar (26.1% v 30.0%, $p = 0.487$). Over the same period OPR for medicated FER remained stable (39.1% after v 42.2% before, $p = 0.285$).

Conclusions: Ongoing pregnancy (>24 w) is significantly more likely if blastocyst transfer occurs on the sixth rather than seventh day after urinary LH surge. NC-FER remains an attractive option for ovulatory women undergoing FER.

P078 Endometrial thickness and its effects on the pregnancy outcomes in hormone replacement frozen thawed embryos transfer cycles

Balakumar Vidhya; Milne Philip; Kini Suresh

Objective: To evaluate the relationship between endometrial thickness (ET) and pregnancy outcomes in hormone replacement frozen thawed embryo transfer cycles (HR FET).

Method: Data on stimulated FET cycles was collected retrospectively for a period of eighteen months between January 2018 to July 2019. This was performed using the local computer database (IDEAS). Total of 427 FET cycles were reviewed. Oral estrogen / Transdermal estrogen patches was used in hormone replacement cycles. Transvaginal ultrasound was performed to measure the maximum endometrial thickness. Pregnancy outcomes including clinical pregnancy rate and pregnancy loss rate were analysed.

Results: All patients were divided into 3 groups based on the endometrial thickness, Group A (<7mm), Group B (7-10mm) and group C (>10mm). A total of 427 cycles were conducted during the eighteen months, of which 410 were hormonally stimulated cycles. Overall pregnancy rate of 55% was achieved. Majority of the pregnancy was noted in the Group B (7-10mm). Among this, the total clinical pregnancy rate was 70.9%, with a miscarriage rate of 28.7% and ectopic pregnancy rate was 0.4%. The clinical pregnancy rate was also highest in Group B (46%), followed by Group C (19.3%) and Group A (5.2%). Interestingly, miscarriage and biochemical loss rate was least in Group A, and maximum in Group B (16.7% miscarriage, 4.8% biochemical loss).

Conclusion: Although the pregnancy loss rate (miscarriage and biochemical pregnancy loss rate) was lower in Group C (>10 mm) compared to Group B (7-10 mm), the clinical pregnancy rate was highest in Group B (7-10mm). This contrary to the studies supporting improved clinical pregnancy outcomes with increasing ET (>10mm). Endometrial thickness is a valuable tool however its role as a surrogate marker for improved outcomes is questionable. More research is needed in this area.

P079 Is assisted reproduction treatment outcome influenced by the time interval from oocyte recovery to ICSI?

Montgomery Sue¹; Birt Katie²; Campbell Alison³; Duncan Colin²; Howles Colin²

¹CARE Manchester; ²University of Edinburgh; ³CARE Fertility

Introduction: It has been demonstrated that a preincubation time of 2-6 hours improves outcome in ART, but there is no evidence available for the optimal preincubation time prior to ICSI. It has recently been suggested that a combination of patients age and in vitro ageing following OR impacts oocyte viability especially in older women whose gametes are more sensitive to prolonged culture. One study found that utilising donor oocytes (age ≤ 35), OR-ICSI time did not impact outcome, but a separate study in which the patient's own oocytes were used (mean age 38) found significant decreases in biochemical and clinical pregnancy rate with increasing OR-ICSI time.

Methods: Anonymised data from 15,561 patients undergoing treatment within one fertility clinic group between 2010-2017 was retrospectively analysed. Patients were grouped due to age (<35 or ≥ 35), OR-ICSI time interval (<4 and >4) and according to the median and upper 75% quartile of number of oocytes retrieved (9 and 13). Main outcomes included clinical pregnancy rate (FHB at 7 ± 1 weeks), live birth and miscarriage rate. All data was analysed using Kruskal-Wallis with Post-hoc testing - Dunn's multiple comparisons test to determine statistical significance.

Results: For women <35 years, OR-ICSI time did not impact clinical outcome. Women ≥ 35 years with median number of oocytes collected (n=9) and OR-ICSI time >4 hours had a significantly lower live birth rate (23%) and clinical pregnancy rate (33%) when compared with all groups of women <35 years (CP 48%-56%; LB 39%-46%). Women ≥ 35 years with the upper quartile (13) oocytes retrieved showed a similar clinical pregnancy rate (46% when OR-ICSI time ≤ 4 hours, 41% when time >4 hours) compared to women <35 years. However, in women ≥ 35 years there was a significant decrease in live birth rate in both OR-ICSI time interval groups (35% and 32%) when compared with women <35 years with the upper quartile (13) oocytes retrieved and OR-ICSI time >4 hours (46%).

Conclusions: The results suggest that women ≥ 35 (especially those with fewer oocytes) warrant a shorter OR-ICSI interval to improve the clinical outcome and therefore the retrieved oocytes from these patients should be prioritised in the lab to undergo ICSI within 4 hours.

P080 Can artificial oocyte activation using calcium ionophore improve blastulation rates in women with previous failed or low blastulation yield?

Avo Santos Margarida¹; Castro Iria¹; Page Alexandra¹; Tozer Amanda¹; Montgomery Sue²; Campbell Alison³

¹CARE Fertility London, UK; ²CARE Fertility Manchester, UK; ³CARE Fertility, UK

Introduction: Artificial oocyte activation (AOA) with calcium (Ca²⁺) ionophore has been shown to improve ICSI outcomes for patients with previous failed or low fertilisation. Intracellular Ca²⁺ release and oscillation, which can be induced artificially, is essential for both oocyte fertilisation and subsequent embryo development. It has been suggested that AOA with Ca²⁺ ionophore may improve embryo development in patients with a history of failed or low blastulation yield.

Methods: Retrospective multicentre preliminary analysis evaluating embryo development outcomes, following risk assessment, validation planning and patient consent. Patients included had been offered ICSI and AOA following a previous cycle (IVF or ICSI) with zero to low blastulation rate. AOA was performed by briefly exposing injected oocytes to the ionophore GM508 Cult-Active (Gynemed, Germany) before in vitro culture in standard culture media. We analysed embryo development outcomes from 12 patients, comparing results from their AOA cycle with their previous (non-AOA) cycle. Primary outcome was blastulation rate on day 5/6.

Results: A total of 176 and 172 oocytes were inseminated in the non-AOA and AOA groups of these 12 patients, respectively. Fertilization rate was increased in the AOA compared with the control group (57 vs 74%), as previously reported. Even though cleavage rate was similar (94 vs 93%), a marked blastulation rate increase was observed (23 vs 43%). We also report an increase in the number of top-quality blastocysts (7 vs 16%). Pregnancy rates were not evaluated due to the small number of patients included and the preliminary nature of this analysis.

Conclusion: AOA with Ca²⁺ ionophore treatment after ICSI resulted in the improvement of embryo development to blastocyst in patients with a history of poor embryo development outcome. This suggests that besides poor fertilisation outcomes, compromised embryo development may be an additional indication for Ca²⁺ ionophore treatment, for this selected group.

P081 PH and osmolality in extended culture systems

Knaggs Paul; Thomas Victoria; Storey Anna; Rennison Kathleen; Middleton Eleri; Evans Sarah

Wales Fertility Institute

Recent developments within the IVF have been increasingly focused on undisturbed, extended embryo culture with many authors reporting improved outcomes when adopting this methodology. Great efforts have been taken to eliminate potential stressors such as changing in temperature and pH with perhaps less focus on osmolality of culture systems based on the assumption that culture oil is impermeable to water vapour. This study aims to investigate pH and osmolality changes in both microdrop and timelapse culture systems over an extended period in a clinically relevant manner. Daily pH and osmolality measurements were made in 15 µl, 30 µl and 50 µl drops prepared by underlaying drops in 4 ml of oil in a standard 35mm culture dish. Embryoscope slides were prepared in the standard manner. New unopened bottles of medium and oil were used to prepare dishes and slides. Dishes were cultured in BT37 benchtop incubators, embryoscope slides were cultured in an Embryoscope. All sham culture was performed at 37°C with either 6% CO₂ in air or 5% O₂/ 6% CO₂/ 89% N₂. Several different embryo culture oils were utilised including washed, unwashed, "light" and "heavy" oils and all dishes/ slides were prepared in unheated laminar flow cabinets which were turned off to minimise evaporative effects of airflow. All experiments were performed in triplicate. pH remained stable after an initial equilibration period in both dishes and slides however the osmolality increased in the culture medium of the embryoscope slide to a greater extent than that of the culture dishes. This work indicates despite the best efforts to stabilise the culture environment, osmolality changes can occur in extended culture even in the absence of embryos. It would be wise for laboratories to take this into account when choosing culture media and designing systems in which it is used.

P082 Safety of originator follitropin alfa (GONAL-f) for fertility treatment -- Frequency of OHSS and thromboembolism in the scientific literature and the Merck KGaA safety database

Velthuis Erica¹; Hubbard Julie²; Longobardi Salvatore¹; D'Hooghe Thomas¹

¹Merck Healthcare KGaA; ²EMD Serono Research & Development Institute

Introduction: Follitropin alfa (GONAL-f) was the first recombinant human follicle-stimulating hormone product on the market. Owing to its mechanism of action and the aim of ovulation induction and controlled ovarian stimulation there is a risk for ovarian hyperstimulation syndrome (OHSS). The risk for thromboembolic events is increased by concomitant pregnancy and OHSS.

Aim: To determine the frequency of OHSS and thromboembolism with GONAL-f based on systematic review of published data and the Merck KGaA Safety Database. **Methods:** Reports of OHSS and thromboembolism were obtained from the Merck KGaA Global Safety Database between 20/10/1995 -- 19/10/2018. MEDLINE and Embase were systematically searched to 19/10/2018 for clinical studies using GONAL-f. Outcomes were extracted for the occurrence of OHSS and by grade (mild, moderate, severe, critical) and for the occurrence of thromboembolism.

Results: 16,525,975 treatment cycles were conducted during the search period with 1,110 cases of OHSS and 80 cases of thromboembolic events. Rates of OHSS were 6.7 per 100,000 treatment cycles (0.007%) and thromboembolism 0.48 per 100,000 treatment cycles (0.0005%). Of the 1,110 OHSS cases, 562 (50.1%) were unknown severity; 146 (13%) were mild; 269 (24%) were moderate; and 130 (11.7%) were severe. Eight cases of life threatening OHSS and 3 fatal cases of OHSS were reported; one fatal case had a thromboembolic and OHSS. 2,243 publications were identified of which 45 unique articles were included. 5,186 patients receiving 5,240 treatment-cycles of ovarian stimulation. 272 cases of OHSS (5190 per 100,000 treatment cycles; 5.19%) and 10 cases of severe OHSS (191 per 100,000 treatment cycles; 0.19%) were identified with no cases of fatal OHSS or thromboembolism.

Conclusion: Evaluation of the frequency of OHSS and thromboembolism in the Global Safety Database and from systematic review of literature demonstrate that the risk for these adverse events after ovarian stimulation treatment with GONAL-f is low.

P083 Characterisation of vaginal microbiome and subsequent cytokine response in assisted reproduction cycles and its association with treatment outcome

Mowla Shahriar¹; Xie Jingyi¹; Lee Yun¹; Ben-Nagi Jara²; Bennett Phillip¹; MacIntyre David¹; Khanjani Shirin¹

¹Imperial College London; ²Centre for Reproductive and Genetic Health

Introduction: Microbiota-host interactions influence metabolic activity as well as immune homeostasis and thus pathogenesis. Dominance of vaginal microbiota by bacterial species such as *Lactobacillus crispatus*, is associated with healthy pregnancies. Conversely, highly diverse vaginal communities enriched with potential pathogens are associated with poor reproductive outcomes including miscarriage and preterm birth. In this study we aimed to assess the stability of vaginal microbiome and determine its association with success of reproductive treatments by means of embryo transfer.

Method: In a prospective cohort study of IVF cycles, cervico-vaginal swabs were collected at 6 timepoints throughout treatment. Metatransomic profiling was performed by sequencing the V1-V2 regions of 16S rRNA using an Illumina MiSeq platform. Resulting sequence data was processed using the MiSeq SOP pipeline with Silva bacterial database used for sequencing alignment. Immunological profiling was performed by assessing the expression of 17 pro- and anti-inflammatory analytes using Human Magnetic Luminex assay and detected by Bioplex®200 system.

Results: *Lactobacillus* was the most abundant genus with 64% of swabs identified as being *Lactobacillus* dominant. *Lactobacillus* dominant samples had lower diversity and were associated with higher stability throughout treatment. There was no association between specific taxa and treatment outcome at any of the timepoints studied. Serum E2 increase from pituitary suppression to ovarian stimulation was associated with decreased expression of IGFBP-1, IL-1 β , IL-1ra and IL-8. Clinical pregnancy was associated with an increase in expression of IGFBP-1, IL-1 β and IL-8 at embryo

transfer. Depletion of Lactobacillus genus was associated with increased expression of pro-inflammatory cytokines; notably IL-1 β , IL-8 and TNF- α .

Conclusion: Dominance by Lactobacillus species is associated with vaginal microbiota stability throughout fertility treatment. Our results indicate that a lack of relationship exists between bacterial taxa and successful treatment outcome at all stages of treatment. However successful embryo implantation correlates with increased expression of cervico-vaginal pro-inflammatory cytokines.

P084 Abdominal compartment syndrome due to severe ovarian hyperstimulation syndrome necessitating decompression midline laparotomy-the size of ovaries matters

Vekinis John; Platts Sophie; Cornish Emily; Eagles Natasha; Roberts Nicola; Iskaros Joseph; Olaitan Adeola; Raza Imran; Abdel Aziz Tarek; Yasmin Ephra

University College London Hospital

Background: Ovarian hyperstimulation syndrome (OHSS) is a potentially fatal iatrogenic complication of increasingly performed controlled ovarian stimulation (COS). Whilst there are a few case reports of abdominal compartment syndrome (ACS) resulting from OHSS in the literature, we present what we found to be the most severe case of this rare complication.

Method: Case presentation Consent was obtained from the patient to present her case for learning. A 37-year-old woman presented to the emergency department with abdominal pain, distension, tachycardia and tachypnoea following oocyte retrieval in another centre the same day. An antagonist protocol with agonist trigger was used but history revealed significant increase in ovarian size after GnRH agonist trigger. The patient developed grade IV intra-abdominal hypertension leading to severe ACS with acute kidney injury requiring intensive care. Ultrasound scan revealed grossly enlarged ovaries with relatively less as it is but significantly high intra-vesical pressure signifying high intra-abdominal pressure.

Result: Paracentesis and abdominal follicular drainage were carried out but did not relieve intra-abdominal pressure or improve symptoms. An emergency decompression midline laparotomy had to be performed which led to improvement in renal function and symptoms. Staggered abdominal wall closure took place over the following week. Multi-disciplinary care was required with input from reproductive medicine, intensive care physicians, general surgeons and specialists in abdominal wall repair, psychologists. GnRH antagonist was used to effect early regression of ovaries. To enable primary closure of the abdomen which was achieved in 10 days.

Conclusions: This may be the most severe case of ACS developing as a consequence of ovarian stimulation. The important lesson is that GnRH agonist trigger does not prevent OHSS. Vigilance is warranted regarding ovarian size as rapid increase can lead to the serious complication of ACS and an awareness of this condition is required in fertility medicine.

Abou Arkoub R1, Xiao CW2, Claman P2, Clark EG3. Acute Kidney Injury Due to Ovarian Hyperstimulation Syndrome. Am J Kidney Dis. 2019 Mar;73(3):416-420. doi: 10.1053/j.ajkd.2018.10.010. Epub 2018 Dec 29.

P085 Is there an effect of season on the fertility of UK dairy cows?

Zeebaree Bayar¹; Kwong Wing²; Gutierrez Carlos³; Sinclair Kevin²; Mann George²

¹Duhok University/College of Veterinary Medicine; ²Nottingham University; ³Universidad Nacional Autonoma de Mexico

While dairy cows are not considered seasonal breeders, in many countries extremes of climate have been shown to influence reproductive performance. The aim of this study was to identify whether such effects can be detected under temperate UK conditions. Cows were housed indoors at the University of Nottingham Dairy Centre (Latitude 52.82° N: Longitude 1.25° W) fed a total mixed ration with concentrates to yield and milked by robots. Conception rate data were collected following 1550 inseminations in a total of 244 Holstein Friesian cows over a 5 year period (Jan 2008 - Dec 2012). Concurrent meteorological data were collected at an adjacent weather station including ambient temperature and

relative humidity and the temperature humidity index (THI) was calculated. Average temperature during the periods 10 days before and 21 days after each insemination were calculated and categorised as $<7^{\circ}\text{C}$, $7 - 15^{\circ}\text{C}$ or $>15^{\circ}\text{C}$. Similarly THIs were calculated and categorised as <40 , $40 - 49$, $50 - 59$ and >60 . Logistic regression was then used to analyse relationships between season, temperature, RHI and conception rate. Conception rate was lower ($P<0.01$) when temperature during the 10 days prior to insemination was $> 15^{\circ}\text{C}$ (29.0%) than when it was $7 - 15^{\circ}\text{C}$ (38.5%) with temperature $< 7^{\circ}\text{C}$ intermediate (33.6%). Average temperature on the day of insemination and during the 21 days following insemination did not affect conception rate. High average THI (>60) during the 10 days prior to insemination was associated with lower ($P<0.05$) conception rate (29.5%) than lower THI (36.5%). Conception rate was significantly ($P<0.05$) higher in spring (40.5%) than winter (33.1%) or summer (32.5%) but not autumn (35.3%). In conclusion conception rate differed between seasons and elevated temperature and THI in the build up to ovulation were associated with reduced conception rate in our temperate climate.

P086 Reproductive outcomes after uterine septum resection

Duncan Megan; Sweeney Eva; McClure Neil

Queen's University Belfast

Background: The septate uterus is associated with adverse reproductive outcomes including recurrent miscarriage, pre-term delivery and fetal malpresentation[1]. Hysteroscopic resection of the uterine septum is a common practice worldwide, aiming to improve reproductive potential.

Objectives: to evaluate the effect of uterine septum resection on reproductive outcomes of women pre- and post-surgery.

Methods: Eighteen subjects underwent hysteroscopic resection of a uterine septum between January 2007 to June 2019. The patients case notes and the regional electronic database were retrospectively reviewed. The following data were collected for each patient pre- and post-resection: total number of pregnancies achieved; number of miscarriages in 1st trimester and 2nd trimester; number of terminations of pregnancy; number of pre-term and term deliveries; method of delivery and other complications related to the pregnancy. In addition, diagnosis and classification of uterine septum; additional fertility factors; indication for resection and intra- and post-operative complications were recorded.

Results: Two subgroups were analysed according to the indication for the resection. Eighteen patients underwent hysteroscopic septal resection, 13 indicated by a history of miscarriage and 5 for treatment of menorrhagia or dysfunctional uterine bleeding. There was one complication of a uterine perforation reported. In the group with a history of miscarriage, the percentage of pregnancies ending in miscarriage was lower after septal resection: pre-resection, 38 out of 47 pregnancies (80.85%) ended in miscarriage compared to 2 out of 14 pregnancies (14.29%) ending in miscarriage post-resection.

Conclusion: This study supports the view that hysteroscopic uterine septum resection improves reproductive outcomes in patients with a history of recurrent miscarriage. Multicentre randomised controlled trials are required in order to determine conclusively the clinical benefits and effectiveness of hysteroscopic septum resection.

1. Venetis CA, Papadopoulos SP, Campo R, Gordts S, Tarlatzis BC, Grimbizis GF 29(6), 665–683. . Clinical implications of congenital uterine anomalies: A meta-analysis of comparative studies.. Reproductive BioMedicine Online 2014; 29(6):665-683.

P087 HABSelect: PICSI equalised miscarriage rates among younger and older women by reducing its incidence in the latter

Miller David¹; West Robert¹; Kirkman-Brown Jackson²; Coomarasamy Arri²; Lewis Sheena³

¹University of Leeds; ²University of Birmingham; ³Queen's University, Belfast

Introduction: The HABSelect randomised clinical trial (1) was designed to test a hyaluronan-based sperm selection platform (PICSI) for treating male infertility and in common with an earlier randomised study (2) reported a significant

fall in miscarriage rates. Herein, we provide evidence from the HABSelect trial data for a strong maternal age effect on this distressing outcome with older women benefiting most from PICSI.

Methods: Couples (N=2,772) were recruited after giving informed consent in 16 independent treatment centres. Following randomisation (PICSI or ICSI), outcome data for 972 couples achieving clinical pregnancy were considered in relation to maternal age and to treatment allocation using contingency tables with risk ratios.

Results: For all clinical pregnancies, miscarriage accounted for 13.0% and 30.5%, respectively of outcomes in younger (≤ 35 ; N=600) and older (> 35 ; N=372) women; RR: 0.49; 95% CI: 0.37,0.66; $p < 0.0001$. Miscarriage accounted for 12.4% and 19.7% of outcomes, respectively, for PICSI (N=485) versus ICSI (N=487) treatment; RR: 0.63; 95%CI: 0.47,0.84; $p = 0.002$. For younger women, rates were 10.3% and 12.7% for PICSI (N=300) and ICSI (N=300) respectively; RR: 0.82; 95%CI: 0.52,1.27; $p = 0.44$. For older women, rates were 15.7% and 31.0% for PICSI (N=187) and ICSI (N=185), respectively; RR: 0.51; 95%CI: 0.33,0.75; $p = 0.0006$. PICSI had no effect on clinical pregnancy rates whether or not data were stratified by age.

Conclusions: Among the clinically pregnant, maternal age was clearly the most significant factor affecting miscarriage rates in HABSelect with older women benefiting most from PICSI treatment. As older couples are increasingly seeking treatment by assisted reproduction (3) and sperm selection had no effect on the establishment of a viable clinical pregnancy irrespective of age. Sperm selection by PICSI may reduce the risk of miscarriages in older women. This finding requires validation in further research.

1. Miller D, Pavitt S, Sharma V, Forbes G, Hooper R, Bhattacharya S, et al. Physiological, hyaluronan-selected intracytoplasmic sperm injection for infertility treatment (HABSelect): a parallel, two-group, randomised trial. *Lancet*. 2019 Feb 2;393(10170):416-22. PubMed PMID: 30712901. 2. Worilow KC, Eid S, Woodhouse D, Perloe M, Smith S, Witmyer J, et al. Use of hyaluronan in the selection of sperm for intracytoplasmic sperm injection (ICSI): significant improvement in clinical outcomes—multicenter, double-blinded and randomized controlled trial. *Hum Reprod*. 2012 Nov 30;28:306-14. PubMed PMID: 23203216. Epub 2012/12/04. Eng. 3. Gleicher N, Kushnir VA, Weghofer A, Barad DH. The "graying" of infertility services: an impending revolution nobody is ready for. *Reproductive biology and endocrinology : RB&E*. 2014 Jul 9;12:63. PubMed PMID: 25012752. Pubmed Central PMCID: 4105876.

P088 Epigenetic (DNA methylation) regulation of gene expression of potassium channels in secretory endometrium of women with recurrent implantation failure (RIF)

AlAhwany Hisham¹; Tufarelli Cristina²; Amer Saad¹; Khan Raheela¹

¹University of Nottingham; ²University of Leicester

Introduction: The endometrium is exposed to constantly changing microenvironment factors, such as pH, lipids, oxygen tension and electrolytes (1) including a concentration of potassium (K⁺) that is 6-fold higher than in plasma (2). Potassium channels in endometrium respond to that high intrauterine fluids potassium concentration and have been shown to play an essential role in implantation, and their altered expression may underlie some instances of infertility. Previous lab work showed that these channels are expressed at different levels in fertile compared to infertile endometrium, but it is unknown whether DNA methylation can account for these changes.

Study question: Are differences in expression of potassium ion channels genes between fertile and infertile human secretory endometrium associated with changes in DNA methylation? Study design, size, duration: This is a lab-based study, comparing the difference in gene expression between normal human secretory endometrium (n=9) and Recurrent implantation failure (RIF) human secretory endometrium (n=7) groups. A new validated protocol was set for the EpiQuik™ tissue methylated DNA immunoprecipitation kit, where a pilot study was done to compare the DNA methylation of the potassium channel genes KCNK9, KCNK10, KCNK17, and KCNMA1.

Material & methods: Human endometrial samples are collected after consenting the patients. Samples are washed from blood in HBSS media. First, RNA is extracted, cDNA synthesized and qPCR carried out. Then, DNA extracted (Qiagen ALLPrep), DNA sheared by Sonicating, Immunoprecipitation (EpiQuik™), qPCR was done using the designed DNA primers (Eurofins©) and SYBR green.

Main results: Potassium channel (KCNK17) was significantly downregulated in infertile mid-secretory endometrium ($P < 0.05$) and the pilot study showed hypermethylation of the KCNK10 and KCNK17 gene in the fertile mid-secretory. Conclusion: The findings from this study provided new knowledge on the role of potassium channels in endometrium, especially KCNK17 that was found to contribute to normal and infertile endometrial function.

NG, K. Y. B., MINGELS, R., MORGAN, H., MACKLON, N. & CHEONG, Y. 2017. In vivo oxygen, temperature and pH dynamics in the female reproductive tract and their importance in human conception: a systematic review. Human reproduction update, 24, 15-34. CLEMETSON, C., KIM, J., DE JESUS, T., MALLIKARJUNESWARA, V. & WILDS, J. 1973. HUMAN UTERINE FLUID POTASSIUM AND THE MENSTRUAL CYCLE. BJOG: An International Journal of Obstetrics & Gynaecology, 80, 553-561

P089 ICSI is associated with lower live birth rates compared to conventional IVF in couples with endometriosis undergoing assisted reproductive treatment: an analysis of 20,326 treatment cycles

Kamali Hajeb¹; Gamaleldin Islam²; Wilson Paul¹; Akande Valentine¹

¹Bristol Centre for Reproductive Medicine; ²North Bristol NHS Trust

Purpose: The effect of endometriosis on IVF outcomes is unclear. Furthermore, no study has evaluated outcomes in conventional IVF versus ICSI in endometriosis patients. The aim of this study is to explore the association between endometriosis and reproductive outcomes and compare the effect of treatment type (IVF versus ICSI) in endometriosis patients undergoing assisted reproductive treatment (ART).

Methods: Anonymised data on all fresh ICSI and conventional IVF treatment cycles performed in the UK from 1991 -- 2016 were retrospectively obtained from the Human Fertility and Embryology Authority (HFEA). Women above 40 years old using donor gametes were excluded. Primary outcome was live birth rates (LBR). Secondary outcomes were fertilisation, implantation and clinical pregnancy rates (CPR). Outcomes were compared in women with endometriosis to women with tubal factor infertility (control). Outcomes were also compared in ICSI versus conventional IVF treatment cycles and further stratified by age group and time period to account for improved LBR over time. Outcomes were adjusted for age, number of embryos created, previous live birth and number of previous IVF cycles.

Results: A total of 120,387 cycles were included. 20,326 cycles had ART for endometriosis compared to 100,061 cycles for tubal factor infertility. Amongst the endometriosis patients, 15,728 had conventional IVF treatment and 4598 had ICSI. The adjusted analysis showed similar fertilisation, implantation, CPR and LBR between endometriosis and tubal factor infertility patients. These findings were consistent across different age groups and different time periods. In endometriosis patients, ICSI was associated with lower LBR (29% vs 32%, OR:0.28 95% CI 0.91-0.98, p<0.041) and CPR (33% vs 39%, OR:0.29 95% CI 0.91-0.98, p<0.025) compared to conventional IVF treatment.

Conclusion: The use of ICSI in couples with endometriosis appears to be associated a lower LBR compared to conventional IVF. Further studies are required to refute or validate these findings.

P090 Enhanced embryo selection encourages SET without impacting on cumulative LBR

Jewitt Sophia; McIntosh Rachel; Wass Catherine; Irving Laura; Keenan Kirsten

Gateshead Fertility Unit

Introduction: The aim of this study was to assess the impact of blastocyst culture with time-lapse enhanced embryo selection on embryo utilisation. The outcome measures were cumulative live birth rate (cLBR) and multiple birth rate (MBR) for patients with cryopreserved embryos.

Methods/Background: A retrospective analysis was performed of all patients with cryopreserved embryos from 2014-2016. The culture system changed during the study period from all cleavage-stage embryos in 2014, to a combination of cleavage-stage and blastocyst-stage embryos in 2015 to all day 5 blastocyst-stage embryos in 2016. All embryos from April 2015 were cultured in the EmbryoScope time-lapse incubator and an enhanced grading scheme was applied.

Outcomes: The outcomes from fresh ET and subsequent FET's from the same cycle were noted. Non-pregnant patient outcomes were categorised as; 1) failed 2) embryos remaining in storage 3) discontinued treatment 4) live birth on subsequent cycles. The cLBR/VEC increased from 58% (2014) to 70% (2015) and 65.5% (2016). The MBR reduced during this time period from 24% (2014) to 5.4% (2015) to 5% (2016). Enhanced embryo selection is reflected in the overall embryo utilisation rate for all patients that reduced from 53% (2014) to 42% (2015) to 36% (2016)

Discussion: The introduction of extended culture with timelapse enhanced embryo selection has reduced the number of embryos used for transfer and cryopreservation and encouraged the practice of single embryo transfer (SET). The results have shown a reduction in the multiple birth rate as patient have been educated as to the benefits of a one-at-a-time strategy, supported by a good cryopreservation programme and the success of fresh plus frozen embryo transfers.

Blennborn M, Nilsson S, Hillervik C and Hellberg D (2005) The couple's decision-making in IVF: one or two embryos at transfer? *Hum Repro* 20, 1292-1297
 McLernon DJ et al (2016) Predicting the chances of a live birth after one or more complete cycles of in vitro fertilisation: population based study of linked cycle data from 113,873 women. *BMJ* 355
 Maheshwari A, McLernon D, Bhattacharya S (2015) Cumulative live birth rate: time for a consensus? *Hum Repro*, 30, 2703-2707
 De Vos A et al (2016) Cumulative live birth rates after fresh and vitrified cleavage-stage versus blastocyst-stage embryo transfer in the first treatment cycle. *Hum Repro*, 31, 2442
 Zdravka Veleva Z, et al. Elective single embryo transfer with cryopreservation improves the outcome and diminishes the costs of IVF/ICSI. *Hum Repro*, 24, 1632-1639,

P091 The effect of autologous Platelet Rich Plasma (PRP) on patients with persistent thin endometrium undergoing frozen embryo transfer cycles

Rao Yogitha

Santasa Fertility Pvt. Ltd

Objective: 'Persistent thin endometrium' is a major challenge in ART. Cycle cancellations are common due to this problem. There are many methods which have come up recently to tackle this challenge. One such treatment involves PRP instillation. The present study was conducted to evaluate the effect of PRP in persistently thin endometrium.
Design: Prospective interventional study

Methodology: Thirty one women who were scheduled for FET and were diagnosed to have persistently thin endometrium were involved in this study. These patients also had cancellation of 2 or more frozen embryo transfer cycles. In addition to HRT with estradiol valerate, 0.5 ml of autologous PRP was instilled into uterine cavity 48-72 hours before progesterone exposure. Endometrial thickness was reassessed by doing transvaginal scan. Frozen embryo transfer was performed when the endometrium reached an optimal pattern in thickness & vascularity.

Results: With this treatment, mean endometrial thickness increased from 5.83 to 7.13 mm. Power Doppler studies showed good vascularity-reaching the zones 3 & 4 of endometrium. Positive beta Hcg was 73.3%. Twenty two pregnancies documented.

Conclusion: Autologous PRP use in persistently thin endometrium resulted in endometrial expansion and appears reassuring.

1. Chen MJ, Yang JH, Peng FH, Chen SU, Ho HN, Yang YS. Extended estrogen administration for women with thin endometrium in frozen-thawed in vitro fertilization programs. *J Assist Reprod Genet.* 2006;23:337-342. 2. Garcia-Velasco JA, Acevedo B, Alvarez C, Alvarez M, Bellver J, Fontes J, et al. Strategies to manage refractory endometrium: state of the art in 2016. *Reprod Biomed Online.* 2016;32:474-89. 3. Kasius A, Smit JG, Torrance HL, Eijkemans MJ, Mol BW, Opremeier BC, et al. Endometrial thickness and pregnancy rates after IVF: a systematic review and meta-analysis. *Hum Reprod Update.* 2014;20:530-41. 4. Chang Y, Li J, Chen Y, Wei L, Yang X, Shi Y, et al. Autologous platelet-rich plasma promotes endometrial growth and improves pregnancy outcome during in vitro fertilization. *Int J Clin Exp Med.* 2015;8:1286-90. 5. Mahajan N, Sharma S. The endometrium in Assisted reproductive technology: how thin is thin.? *J Hum Reprod Sci.* 2016;9:3-8. 6. Takasaki A, Tamura H, Miwa I, Taketani T, Shimamura K, Sugino N. Endometrial growth and uterine blood flow: a pilot study for improving endometrial thickness in the patients with a thin endometrium. *Fertil Steril.* 2010;93:1851-8. 7. Gleicher N, Kim A, Michaeli T, Lee HJ, Shohat-Tal A, Lazzaroni E, et al. A pilot cohort study of granulocyte colony-stimulating factor in the treatment of unresponsive thin endometrium resistant to standard therapies. *Hum Reprod.* 2013;28:172-7. 8. Nagori C, Panchal S. Endometrial vascularity: its relation to implantation rates. *Int J Infertil Fetal Med.* 2012;3:48-50. 9. Aghajanova L, Houshdaran S, Balayan S, Irwin J, Huddleston H, Giudice L. Platelets for endometrial regeneration: a novel approach. *Fertil Steril.* 2016;106:e82. 10. Richter KS, Bugge KR, Bromer JG, Levy MJ. Relationship between endometrial thickness and embryo implantation, based on 1,294 cycles of in vitro fertilization with transfer of two blastocyst-stage embryos. *Fertil Steril.* 2007;87:53-59.

P092 The impact of endometrioma on IVF/ICSI reproductive outcomes: a systematic review and meta-analysis

Alshehre Sallwa¹; Fenwick Mark¹; Narice Brenda²; Metwally Mostafa²

¹The University of Sheffield; ²Sheffield Teaching Hospitals

Background and objectives: Endometriosis has a detrimental effect on fertility(1). Females with endometriosis and the presence of endometrioma may require assisted reproductive techniques to conceive(2). The specific influence of endometrioma on the reproductive outcome of females undergoing in vitro fertilisation (IVF) or intracytoplasmic sperm injection (ICSI) is controversial(3). This review aimed to determine the effect of endometrioma on IVF/ICSI outcomes.

Methods: A systematic review with electronic searches of PubMed and Web of Science (BIOSIS, MEDLINE) up to September 2019 was conducted to identify articles examining females who have endometrioma and are undergoing IVF/ICSI. Eight studies were included for meta-analysis. The primary outcome was the live birth rate (LBR). Secondary outcomes were clinical pregnancy rate (CPR), the number of oocytes retrieved, the number of metaphase II (MII) oocytes retrieved, the number of embryos and top-quality embryos, implantation rate (IR), the duration of stimulation and Gonadotropin dose. Results The data were pooled using the RevMan software by the Cochrane Collaboration. Heterogeneity between studies was based on the results of the I² statistics. A random-effect model was used for high heterogeneity between studies. The number of oocytes (weighted means difference [WMD]-2.25; 95% CI-3.34 to -1.06, P<0.001) and the number of MII oocytes retrieved (WMD-4.64; 95% CI-5.65 to -3.63, P<0.00001) were significantly lower in women with endometrioma than women without. The duration of stimulation was significantly higher in women with endometrioma than women without (WMD 1.53; 95% CI-0.98 to 1.80 P<0.00001). The total number of embryos and top-quality embryos, IR, CPR, LBR and the Gonadotrophin dose, were similar. Conclusions Women with endometrioma undergoing IVF/ICSI had similar reproductive outcomes compared with women without. Nevertheless, endometrioma patients have a lower mean number of oocytes, MII oocytes retrieved and higher duration of ovarian stimulation, which suggests that the presence of the endometrioma could affect the ovarian function.

References 1- Meuleman C, Vandenabeele B, Fieuws S, Spiessens C, Timmerman D, D'Hooghe T. High prevalence of endometriosis in infertile women with normal ovulation and normospermic partners. *Fertility and sterility*. 2009 Jul 1;92(1):68-74. 2- Opøien HK, Fedorcsak P, Omland AK, Åbyholm T, Bjercke S, Ertzeid G, Oldereid N, Mellembakken JR, Tanbo T. In vitro fertilization is a successful treatment in endometriosis-associated infertility. *Fertility and sterility*. 2012 Apr 1;97(4):912-8. 3- Hamdan M, Dunselman G, Li TC, Cheong Y. The impact of endometrioma on IVF/ICSI outcomes: a systematic review and meta-analysis. *Human Reproduction Update*. 2015 Jul 12;21(6):809-25

P093 The addition of recombinant LH or HMG to recombinant FSH in anticipated poor responders does not improve live birth rates

Balfoussia Danaï; Saravelos Sotirios; Manukian Barbara; Jansa Perez Marta; Rai Raj; Salim Rehan

Imperial College Healthcare NHS Trust

Background: There is ongoing controversy regarding the benefits of LH in controlled ovarian stimulation regimes in poor responders. The aim of our study was to compare rFSH monotherapy to rFSH with hMG, and rFSH with rLH.

Methods: Retrospective analysis of consecutive IVF/ICSI cycles in a large teaching hospital between January 2014 and January 2018 comparing rFSH monotherapy (Group 1), rFSH with hMG (Group 2) and rFSH with rLH (Group 3) in anticipated poor responders, defined as starting dose of rFSH≥300IU.

Results: A total of 1016 cycles were analysed (Group 1: 541, Group 2: 224 and Group 3: 250). Women in Group 1 were marginally younger compared to group 3 (36.5±3 vs 37.6±3.5). AFC was comparable across all groups. Duration of ovarian stimulation was longer in Group 2 [(12±2 days vs 11±2 days in Groups 1 and 3), p<0.05]. The oocyte yield was greater in Group 1 [(9±5 vs 7±4 and 7±4), p<0.001] but with no difference in maturity rates [(81%, 82% and 82%), p=0.918] or fertilisation rates [(72%, 75% and 75%), p=0.391]. There was no difference in the number of embryos transferred [(1.6±0.5 v 1.6±0.5 v 1.7±0.5), p= 0.187] or in clinical pregnancy [(29%, 30% vs 29%), p=0.951]. Live birth rates were comparable [(24%, 24% vs 21%), p=0.593] even after controlling for age differences. Group 3 had fewer good quality cryopreserved embryos compared to Group 1 and 2 [(0.3±0.9 v 0.6±1.3 and 0.8±1.5), p<0.005]. The live birth rate in subsequent FET cycles was comparable among all three groups.

Conclusions: The addition of rLH or hMG to ovarian stimulation regimes did not result in higher live birth rates compared to rFSH monotherapy. Cycles using hMG were associated with a longer duration of stimulation with no improvement in clinical outcomes. Further evaluation with RCTs is recommended to validate these results.

P094 The use of hMG in expected good responders does not result in higher live birth rates

Balfoussia Danai; Saravelos Sotirios; Sarodey Geet; Jansa Perez Marta; Rai Raj; Salim Rehan

Imperial College Healthcare NHS Trust

Background: There is ongoing debate around the optimal gonadotrophin for ovarian stimulation in women with good ovarian reserve. We aimed to compare rFSH versus hMG monotherapy.

Methods: Retrospective analysis of consecutive IVF/ICSI cycles in a large teaching hospital between January 2014 and January 2018 comparing hMG (Group 1) and rFSH (Group 2) monotherapy in anticipated good responders, defined as starting dose of FSH 150 IU.

Results: A total of 632 cycles were analysed (Group 1: 76 and Group 2: 556). The two groups were comparable in terms of age, antral follicle count, choice of protocol and method of oocyte fertilisation. Duration of ovarian stimulation was longer in Group 1 [(12±2 vs 11±2 days), $p<0.001$]. The need for busarelin trigger for risk of OHSS was comparable between the two groups (16% vs 28%, $p=0.073$). The oocyte yield and oocyte maturity were greater in Group 2 [(15±8 vs 12±6, $p<0.001$) and (86% vs 81%, $p=0.046$) respectively]. Fertilisation rate, day of embryo transfer, number of embryos transferred and embryo quality were comparable in both groups. There was no statistical difference in clinical pregnancy (43% vs 45%, $p=0.777$) and live birth rate (42% vs 42%, $p=0.855$). The number of high quality embryos available for cryopreservation was comparable in the two groups (3±3 vs 3±4, $p=0.804$). Of those who did not conceive in the fresh cycle, 175 underwent a FERC (Group1=25, Group2=150). There was no difference in clinical pregnancy and live birth rates [(52% vs 51%, $p=0.951$) and (48% vs 49%, $p=0.951$)].

Conclusions: We report no significant difference in clinical outcomes. Our data shows that rFSH monotherapy resulted in shorter duration of treatment and higher oocyte yield with no additional significant risk of OHSS. Our findings are limited by the observational non-randomised study design.

P095 The impact of maternal age on target gene expression in embryos and reproductive organs

Thoma Juliane; Schindler Maria; Pendzialek Mareike; Seeling Tom; Jung Juliane-Susanne; Navarrete-Santos Anne

Department of Anatomy and Cell Biology, Martin Luther University Halle-Wittenberg

An advanced maternal age is associated with subfertility, pregnancy complications and worse pregnancy outcome. We hypothesize that maternal age affects the expression of key factors of embryo-maternal communication in embryos and supplying uterine tissue during preimplantation period. To study effects of maternal age on early embryonic development, we obtained preimplantation blastocysts and endometrium from young (16 weeks) and old rabbits (over 108 weeks) on day six post coitum. We investigated the expression of target genes directly associated with cellular age and stress (like SIRT1, PTEN, ATG7 and ATF3) as well as metabolic marker genes (e.g. PPAR, CREB, FASN). Expression was quantified on mRNA and protein level by quantitative RT-PCR and Western Blot, respectively. SIRT1, PTEN and ATG7 were lower expressed, correlating with a reduced CREB phosphorylation and ATF3 protein amounts in endometrium and blastocysts of old rabbits. The expression of insulin like growth factors (IGF) and insulin receptor were depending on maternal age, indicating an age related alternation of growth factor expression in the preimplantation period. Metabolic target genes like FASN, PPAR α and PPAR γ were affected at the mRNA level, only. In order to understand regulatory mechanisms we quantified target genes responsible for micro-RNA-processing (Drosha), DNA-methylation (DNMT3a) and transcription factors, WNT2 and OCT4. All regulatory target genes were downregulated in embryos from old rabbits. In conclusion, results demonstrated that maternal age influences endometrial and embryonic target gene expression, indicating that age-related changes have consequences for the embryo-maternal interaction during preimplantation. This gene expression changes may be crucial for decreased fertility at higher maternal age.

P096 Fertility outcomes in IVF/ICSI cycles with low Anti-Müllerian hormone

Background: Anti-Müllerian hormone (AMH), produced by developing follicles in the ovary, is used as a marker of low ovarian reserve. A low AMH signifies a diminished egg reserve and a reduction in ovum quality. AMH tests are being routinely offered to patients at NHS trusts at the cost of £100, therefore it is important for patients to be able to assess whether AMH is an accurate marker of fertility. The primary aim of this study was to investigate the relationship between AMH and fertility outcomes so that we could provide women with low AMH levels better counselling if they choose to undergo IVF/ICSI treatment.

Methods: This was a retrospective observational study undertaken between April 2015 and March 2019. The BWFC fertility treatment database was used to identify all women with an AMH<5pmol/l who underwent fresh IVF and ICSI cycles. The primary outcome was clinical pregnancy rate (CPR).

Results: A total of 117 IVF cycles (n=117) undertaken by 101 women were identified as having an AMH<5pmol/l. The mean number of eggs collected per cycle was 4.79. The percentage of cycles resulting in an embryo transfer was 74% (86/117) and the percentage ending in a day 5 blastocyst transfer was 10% (12/117). The percentage of cycles achieving a positive urinary pregnancy test was 30% (35/117) and the CPR was 15% (17/117). Data for live birth was not yet available for some patients and was therefore not included in this study. A subgroup analysis identified women with an AMH<2pmol/l as having a CPR of 7%.

Conclusion: The study suggests that low AMH levels can be used as a marker for reduced fertility outcomes, and that women with an AMH<2pmol/l may require more specific counselling based on their significantly lower CPR.

P097 The influence of maternal BMI on early embryo development and offspring birth weight by using a consistent paternal contributor

Mason Laura; Leary Christine; Maguiness Steve D

Hull IVF Unit

Aim: To examine the maternal contribution to early embryonic development (prior to embryonic genome activation) and offspring birth weight in a study population, conceived using a single sperm donor.

Method: A retrospective data analysis based on one sperm donor with a BMI of 24.7Kg/m² and 23 recipients of which 10 families were created and 13 babies fathered.

Significance: There was no significant relationship between the number of top grade embryos on day 3 (≥Grade-3) and female age (P=0.3) or BMI (P=0.21). However, pregnancy outcomes demonstrate a difference with BMI; non-pregnant patients (12/23) had a significantly higher BMI than those who achieved a pregnancy (28.8 and 24.1 Kg/m² respectively) P<0.05. This implies that the maternal BMI may subtly influence embryo viability and implantation potential independent of the paternal contribution to embryogenesis. This is further supported by the finding that embryo development for this study cohort was comparable to that of patients treated using partner sperm during study period (66/101 65.3% Donor-IVF and 718/1251 57.4% Partner-IVF ;P=0.14). A secondary outcome measure was to analyse female BMI (range 19.9-32.4Kg/m²) to birth weight (range 2100-4338grams), which shows an R² value of 0.11, P=0.26, inferring that there is a poor relationship between female BMI and baby birth weight and paternal genetic contribution could be an overriding influencing on birth weight. However, there are many confounding variables likely to influence this outcome.

Conclusion: Maternal BMI influences embryo viability beyond observable embryo grading criteria, independent of paternal factors. However the paternal contribution to birth weight of offspring may be more important than previously suspected and merits further analysis.

Wider implications: Few studies have looked at the differential maternal and paternal contributions to birth outcomes and a broader understanding of the paternal contribution to embryo viability can be used to counsel patients when opting for donor gametes.

P098 AMH predicts cumulative ovarian performance better than individual cycle performance

Papathanasiou Thanos; Stock Donna; Curry Andrea

Bourn Hall Clinic

Background: AMH is routinely used for predicting ovarian response before the 1st IVF. However, little is known about whether AMH can predict beyond the 1st cycle.

Methods: Consecutive women undergoing ovarian stimulation and oocyte collection between July 2013 up to April 2018 were included. A single random AMH measurement was taken from every woman just before the 1st cycle. The AMH assay (Beckman-Coulter, Gen II) was consistent during the period of study.

AMH levels were correlated with ovarian performance during the 1st cycle, the 2nd cycle as well as the average of the two cycles. Three parameters of ovarian performance were assessed: antral follicle count (AFC) at baseline, number of recruited follicles (>10mm) on trigger day and number of retrieved oocytes.

Non-parametric correlation (R) coefficients were compared between dependent samples, with Bonferroni adjustment for multiple comparisons.

Results: Approximately 2123 women underwent at least 1 collection, out of whom 809 women also underwent a 2nd collection. Most women (84%) had their 2nd collection within a year.

AMH predicted ovarian performance comparably for the 1st and the 2nd cycle.

However, AMH was better at predicting the average ovarian performance of the two cycles than for each cycle alone (AFC: Rave=0.78 vs R1=0.72 $p<0.001$, Rave=0.78 vs R2=0.71 $p<0.001$ / Recruited follicles: Rave=0.69 vs R1=0.60 $p<0.001$, Rave=0.69 vs R2=0.62 $p<0.001$ / Retrieved oocytes Rave=0.51 vs R1=0.45 $p<0.001$, Rave=0.51 vs R2=0.45 $p<0.001$).

Sensitivity analysis including only women with 2 collections did not change the above findings.

Conclusions: We demonstrated for the 1st time that AMH provides a holistic prediction of ovarian performance that extends beyond the 1st IVF cycle. This implies that if ovarian response during the 1st cycle is higher or lower than predicted, performance during the subsequent cycle should compensate for this.

P099 Stimulation outcomes in age versus non age induced poor ovarian reserve (AMH<5.4pmol/L)

Antwi-Ahima Gloria; Choudhary Meena

Newcastle Upon Tyne Hospital

Background: Poor ovarian reserve (POR) is the decline in oocyte pool and is associated with poor stimulation response, high cancellation rate and low pregnancy rate. It is a physiological process with advancing maternal age however for some women, this process is accelerated.

Aim: To determine whether the stimulation outcome in young women with POR is better than that of older women with POR by comparing the number of oocytes, maturation rate and fertilization rate in women over 38 years old with AMH of <5.4pmol/L to that of young women under 35 years with AMH <5.4pmol/L.

Methodology: Retrospective study looking at women going through their first cycle of IVF/ICSI with AMH <5.4pmol/L and under 35 years or over 38 years reaching the oocyte collection stage from January 2016 to May 2019 at a tertiary hospital in the United Kingdom. There were 32 patients in the young group (group A) and 28 in the older group (group B).

Results: The average oocyte number was 4.4 ± 3.22 in group A and 4.1 ± 2.78 in group B. The average percentage oocyte maturation was 92.5% for group A versus 85.7% for group B. The average percentage of normal fertilization was 70.4% for group A versus 71.5% for group B. 26 women out of 32 had embryo transfer in group A while 24 out of 28 had embryo transfer in group B. 7 women had a clinical pregnancy in group A versus 4 in group B giving a pregnancy rate of 26.9% and 16.6% respectively.

Conclusion: Women under 35 years and those over 38 years with $AMH < 5.4 \text{ pmol/L}$ who reach oocyte collection stage of treatment have comparable outcomes of ovarian stimulation however pregnancy rate per embryo transfer is likely to be better in the younger group.

P100 Price comparison of AMH and follicular phase gonadotrophins for testing ovarian reserve

Cuffolo Romana; Husain Fatima; Shenasan Goli; Azar Bardia; Grenshaw Dawn

Wexham Park Hospital

Background: AMH (Anti-Mullerian Hormone) is a new biomarker of ovarian reserve and PCOS. AMH shows minimal inter-cycle and intra-cycle variation and is superior in determining ovarian function compared to follicular phase FSH/LH/E2 (1). Due to its cost, AMH is mainly used in women prior to starting IVF treatment. We decided to compare the cost effectiveness of AMH testing versus the conventional method of timed hormone tests.

Methods: We calculated the cost of universal AMH testing compared to current timed hormone tests for all women aged 16-51 who had ovarian reserve and ovulatory status tested in our unit in 2016. The cost (including phlebotomy) of AMH is £21 and the total cost of follicular FSH/LH/E2 and mid-luteal progesterone together is £10.35. The gynaecology clinic appointment costs £142 (2).

Results: 8124 women were included. Most women had correctly timed blood tests but there were 1104 women who had FSH/LH/E2 and progesterone tested on the same day, which needed repeating as some or all of these tests were mistimed. 328 AMH tests were performed, 231 women had AMH as well as gonadotrophins tested. The total cost of blood tests in our study was £61678.37. We estimated that 773 extra gynaecology clinic appointments were needed to check the results of the incorrectly timed or additional blood tests, increasing the total cost to £171444.37. The cost of universal AMH testing in this cohort would be £170604, which could save £840.37 per annum. This excludes the cost of repeat appointments for blood tests in primary care and the financial cost to the patients.

Conclusions: A single blood test for AMH could improve the detection of poor ovarian reserve and PCOS and potentially save time and cost for the woman and her clinician.

(1) Visser JA, Themmen AP, Anti-mullerian hormone and folliculogenesis. *Mol cell endocrinol* 2005; 234: 81-86 (2) NHS Reference Costs. 2017-2018. <https://improvement.nhs.uk/resources/reference-costs/#rc1718>

P101 Immunohistochemical localization of androgen receptors in anacyclus pyrethrum roots methanolic extract treated male rats

Riaz Muhammad¹; Shahid Muhammad²; Robinson Jane³

¹University of Sargodh, Pakistan; ²University of Agriculture, Faisalabad, Pakistan; ³Institute of Biodiversity, Animal Health and Comparative Medicine, University of Glasgow

Background: Androgens are pivotal for maintaining the normal spermatogenesis and exert their effects through androgen receptors (AR) in tissues. To understand the mode of AR action in testis, it is imperative to determine the cellular distribution of AR in testicular tissue. The current study investigated the distribution of AR in testicular tissue of male rats using anti-rabbit AR polyclonal antibody.

Methods: Adult male rats were divided into six groups (n=5) including normal control (A), Carbon tetrachloride (CCl₄) intoxicated group (B), Testosterone group (C) and three test groups (D, E and F) administered with 50, 100 and 200 mg/Kg bw dose of A. pyrethrum roots methanolic extract for 42 days. Body, testis and relative testis weight of rats were

recorded. Variations in testicular histology of rats were observed through H & E staining. After microwave antigen retrieval, AR immunostaining was detected in the nucleus or cytoplasm of cells in rat testis through immunohistochemistry by Vectastain avidin-biotin complex using diaminobenzidine (DAB) chromogenic substrate.

Results: The results depicted significant ($p < 0.05$) variation in body weight and relative testis weight of rats treated with different doses of A. pyrethrum roots extract compared to control rats. Testicular histology revealed the protective effect of A. pyrethrum roots against CCl₄ intoxication. Variations in immunostaining of AR in testicular tissue were observed among different study groups. Significant improvement in AR immunostaining in the nucleus or cytoplasm of cells in testicular tissue was observed in A. pyrethrum roots extract administered rats compared to CCl₄ intoxicated rats. CCl₄ intoxication disrupts the AR in testicular cells resulting in less or poorly stained cells.

Conclusion: We concluded that A. pyrethrum roots extract has androgenic potential which leads to the increased AR immunostaining in testicular cells and might enhance male fertility.

P102 The effect of different luteal phase support (LPS) regimes on pregnancy outcomes in FET cycles

Spofforth Katherine¹; Shehadeh Zachariah²; Zaki Mona²; Jose K²; Kalra Gurpreet²; D'Angelo Arianna²

¹Cardiff University Medical School; ²Wales Fertility Institute

Objective: To compare the effectiveness of two different forms of progesterone supplementation Lubion (SC)(subcutaneous) and Cyclogest (PV/PR)(Vaginal/ rectal), after Frozen Embryo Transfer (FET) at the Wales Fertility Institute (WFI). The primary outcome is Clinical Pregnancy Rate (CPR). Secondary outcomes include biochemical pregnancies, ectopic pregnancies and miscarriages.

Methods: Retrospective audit of data collected from patient notes and WFI database on FETs between January 2017 and March 2019. Women were given either Lubion 25mg twice a day (SC) or Cyclogest 400mg twice a day (PV/PR)

Results: Data from 348 cycles of FET were collected and analysed. The CPR was greater amongst Lubion cycles (27.94%) than Cyclogest cycles (25.24%). EPR was greater in Cyclogest cycles (2.90%) than Lubion (1.29%). BPR was greater in Lubion cycles (23.38%) than in Cyclogest (21.74%). Furthermore, Lubion cycles had a greater miscarriage rate both per positive pregnancy test (9.09% as opposed to 8.70% with Cyclogest) and per CPR (12.28 as opposed to 11.76% with Cyclogest).

Conclusions: Clinical pregnancy rate was greater amongst Lubion assisted cycles than in Cyclogest cycles. Furthermore, Lubion cycles had a lower ectopic pregnancy rate. Cyclogest cycles were found to have a lower rate of miscarriages and biochemical pregnancies; however, the difference was minimal. These results suggest that Lubion is a more effective form of progesterone supplementation than Cyclogest but no firm conclusions can be drawn as none of the results were statistically significant. Further data collection is required to provide more robust evidence for differences in outcomes between different forms of progesterone supplementation.

References: 1. Wade J, MacLachlan V, Kovacs G. The success rate of IVF has significantly improved over the last decade. Australian and New Zealand Journal of Obstetrics and Gynaecology. 2015;55(5):473-476. Available from: <https://obgyn.onlinelibrary.wiley.com/doi/abs/10.1111/ajo.12356> 2. Gazvani R, Russell R, Sajjad Y, Alfirevic Z. Duration of luteal support (DOLS) with progesterone pessaries to improve the success rates in assisted conception: study protocol for a randomized controlled trial. Trials. 2012;13:118. 3. Liu X, Mu H, Shi Q, Xiao X, Qi H. The optimal duration of progesterone supplementation in pregnant women after IVF/ICSI: a meta-analysis. Reproductive Biology and Endocrinology. 2012;10(1):107. Available from: <https://www.ncbi.nlm.nih.gov/pubmed/23237065> 4. PROGESTERONE | Medicinal forms | BNF content published by NICE. Bnf. nice. org. uk. 2019. Available from: <https://bnf.nice.org.uk/medicinal-forms/progesterone.html> 5. 13. Study design and choosing a statistical test | The BMJ. Bmj. com. 2019. Available from: <https://www.bmj.com/about-bmj/resources-readers/publications/statistics-square-one/13-study-design-and-choosing-statistics> 6. Chi Square Calculator 2x2 (includes Yates correction). Socscistatistics. com. 2019. Available from: <https://www.socscistatistics.com/tests/chisquare/default2.aspx>

P103 Relaxin receptors and their functionality in the ruminant

Alhujaili Waleed; Dai Y; Ivell R; Anand-Ivell R

Background: In most mammals the structurally related GPCR receptors, RXFP1 and RXFP2, form endocrine dyads with their specific ligands, the peptide hormones relaxin and INSL3, respectively. Whilst both peptide hormones can bind and activate only their cognate receptors in the physiologically low nanomolar range, in the human, relaxin can also interact with RXFP2 but only at high, supraphysiological levels. In the female mammal, relaxin is important in a range of reproductive physiologies including implantation, decidualisation, uterine vascularisation and quiescence, birth, lactation, and maintaining systemic osmolarity during pregnancy [1]. We have recently confirmed by synteny analysis that the relaxin gene in ruminants (cows, sheep), though not the INSL3 gene, has been deleted [2]. Aims. The present study was undertaken to (a) confirm and characterise the ruminant receptors RXFP1 and RXFP2, (b) determine whether these receptors are still functional in the female ruminant reproductive tract, and thus (c) whether alternative RXFP1 ligands might offer interventional possibilities to reduce the high infertility of the dairy cow.

Methods: The bovine genes for RXFP1 and RXFP2 were cloned, transiently expressed in HEK293T cells, and analysed in vitro. Multiplexed RT-PCR analysis was devised to determine RXFP1 and RXFP2 expression (and possible splice variants) in diverse primary cells and tissues of the female bovine reproductive tract, as well as in the ovine uterine cell-lines oGE, oLE and oS, representing glandular and luminal epithelium, and stromal cells, respectively. Cells were also cultured to determine the functionalities of the naturally expressed receptors.

Results and Discussion: In vitro expression of transfected bovine RXFP1 and RXFP2 receptors, showed that whereas the latter, like its human counterpart, responded to INSL3 in the low nanomolar range by increasing cAMP production, the activity of RXFP1 was evidently attenuated, responding to porcine relaxin only at high concentration (ca. 50nM). Apparently full-length RXFP1 gene transcripts were

References: [1] Anand-Ivell R, Ivell R. (2014) Regulation of the reproductive cycle and early pregnancy by relaxin family peptides. *Mol Cell Endocrinol* 382: 472-479. [2] Dai Y, Ivell R, Liu X, Janowski D, Anand-Ivell R (2017) Relaxin-family peptide receptors 1 and 2 are fully functional in the bovine. *Front Physiol* 8: 359.

P104 Regulation of Dual Specificity Phosphatases (DUSPs) by fibroblast growth factor 2 in bovine granulosa cells

Relav Lauriane; Abulghasem El Arbi; Zhongliang Jiang; Bourmaud Violaine; Price Christopher

University of Montreal

Intra-ovarian factors, such as fibroblast growth factor-2, regulate folliculogenesis. FGF2 stimulates proliferation of granulosa cells through MAPK, PKC and AKT pathways. A rapid and transient increase in MAPK3/1 phosphorylation is a typical mitogenic signal, and the intensity and duration of phosphorylation is regulated by DUSPs (1). In rats, DUSPs are implicated in the dephosphorylation of MAPK3/1 after FSH stimulation (2), but the regulation of their expression upon FGF signaling in the ovary is unknown. The aim of the present study was to identify which DUSPs are controlled by FGF2 and to determine the pathways involved. Bovine granulosa cells were cultured under serum-free conditions and treated with FGF2 and/or specific inhibitors targeting MAPK, PKC and AKT pathways. Abundance of mRNA encoding DUSPs was assayed by RT-qPCR. Treatment with FGF2 significantly upregulated 3 of 9 DUSPs tested: DUSP5 and DUSP6 mRNA levels increased at all time points (2-6-8 h), and DUSP1 at 2-6 h only. Inhibition of PI3K, involved in AKT pathway, with or without FGF2 treatment did not affect the expression of these 3 DUSPs. Similarly, we did not observe changes in their mRNA levels when targeting PKC. Disruption of calcium signaling with a PLC inhibitor or an ionophore increased basal DUSP1, DUSP5 and DUSP6 mRNA levels but did not further increase FGF2-stimulated mRNA levels. Inhibition of MAPK3/1 decreased FGF2-stimulated DUSP1, DUSP5 and DUSP6 expression. A major transcription factor involved in regulation of FGF-target genes is EGR1; adenoviral overexpression of EGR1 increased DUSP1, DUSP5 and DUSP6 mRNA levels, which not diminished by the presence of MAPK3/1 inhibitor. We conclude that DUSP1, DUSP5 and DUSP6 are target genes of FGF2 and are controlled through calcium signaling and a MAPK3/1 - EGR1 pathway.

1. Patterson KI, Brummer T, O'Brien PM, Daly RJ. Dual-specificity phosphatases: critical regulators with diverse cellular targets. *Biochem J*. 15 mars 2009;418(3):475-89.
2. Donaubauer EM, Law NC, Hunzicker-Dunn ME. Follicle-Stimulating Hormone (FSH)-dependent Regulation of Extracellular Regulated Kinase (ERK) Phosphorylation by the Mitogen-activated Protein (MAP) Kinase Phosphatase MKP3. *J Biol Chem*. 9 sept 2016;291(37):19701-12.

P106 Prevalence of polycystic ovarian syndrome in secondary school girls in Mansoura

Shams Maher¹; Saad Sally²; Shabanna Ahmed¹

¹Faculty of Medicine Obstetric Gynaecology Department; ²Talkha Central Hospital

Backgrounds: Polycystic ovary syndrome (pcos) is very common endocrine disorders that approximately 7% of reproductive age women its heterogeneous disease that usually presents during adolescence.

Aim of the work: To determine the prevalence of polycystic ovary disease in adolescence Study design; cross sectional study was conducted at four secondary schools girls in Mansoura

Methods: The central agency for public mobilization and statistics gave a permission after a security approval to full out in four secondary schools .

Results: The total numbers of students in all four schools is 4873 aged between 15 and 18. girls suffered from hyperandrogenism and chronic ovulation 395. They did hormonal profile FSH, LH and serum prolactin. After reviewing the tests 215 cases with confirmed diagnosis of polycystic ovary syndrome from a total of 2000 students with a percentage of 10.75% Conclusions prevalence of pcos in adolescence vary largely in different population. The prevalence of pcos accurately if they are done in a large number of population hence more studies needed to detect the prevalence accurately especially if they done in a large number of population

P107 Prenatal androgen exposure disturbs female liver phenotype in an ovine model of PCOS

Siemienowicz Katarzyna¹; Furmanska Klaudia¹; Talia Chiara²; Filis Panagiotis²; Fowler Paul²; Duncan Colin³; Rae Mick¹

¹Edinburgh Napier University; ²University of Aberdeen; ³University of Edinburgh

Introduction: Women with Polycystic Ovary Syndrome (PCOS), a common (up to 10% of women of reproductive age) endocrine condition characterised by hyperandrogenemia are at increased risk of developing non-alcoholic fatty liver disease (NAFLD), independent of obesity. Studies suggest prenatal androgen excess as contributory to NAFLD development in women with PCOS, but the underlying mechanisms are unclear.

Methods: Female ovine fetuses were injected with testosterone propionate (TP) or vehicle control (C) at day 62 and 82 of gestation. At adolescence (11 months of age) these offspring were sacrificed (C, n=10; TP, n=15). Hepatic triglyceride (TG) content, plasma TG and free fatty acids (FFA) were measured and compared with transcript expression of genes key to hepatic lipid homeostasis.

Results: Prenatally androgenized females had increased hepatic TG content ($4.03 \pm 1.17 \mu\text{g}/\text{mg}$) as compared to controls ($3.09 \pm 0.66 \mu\text{g}/\text{mg}$) ($P=0.027$), independent of body weight and adiposity. There was no difference in plasma TG (C, 0.31 ± 0.18 vs TP, $0.38 \pm 0.19 \text{ mmol}/\text{l}$; $P=0.37$) or plasma FFA (C, 0.68 ± 0.3 vs TP, $0.82 \pm 0.18 \text{ mmol}/\text{l}$; $P=0.079$). There were no differences in gene expression of hepatic FFA uptake / translocation (SLC27A2, SLC27A5, SLC27A6, CD36, FABP5, CAV1 and CAV2) or de novo fatty acid synthesis (ACACA, FASN, ELOVL4). There was a significant reduction in gene expression involved in mitochondrial beta oxidation (CPT1B; $P=0.0025$), lipid metabolism regulation (FGF21; $P=0.048$) and (TM6SF1; $P=0.012$), and hepatic TG export (APOC3, $P=0.03$).

Conclusion: Prenatal androgen exposure alters hepatic gene expression in adolescent female offspring, predisposing them to hepatic triglyceride accumulation independent of body weight and adiposity.

P108 Case report: Oocyte cryopreservation in Turner syndrome mosaic with polycystic ovaries

Czink Jessica¹; MacDougall Jane²; Prakash Alka²

¹University of Cambridge; ²Cambridge IVF

Turner syndrome (TS) is a congenital disorder of sex development affecting female infants, where an X chromosome is either partially or entirely missing in at least one cell line. Accelerated germ cell apoptosis and increased ovarian fibrosis leads to follicular depletion and gonadal dysgenesis. Polycystic ovaries (PCO) have only been described twice in TS patients. We believe this is the first case to describe oocyte cryopreservation in a patient with TS mosaicism and PCO. Mosaic Turner syndrome with a paternally inherited balanced reciprocal translocation (2:6) was diagnosed via amniocentesis. Postnatal, childhood and pubertal development was uneventful. At 17 years, anti-Müllerian hormone (AMH) was 68pmol/L. At 19, her cycles were 31-38 days. Cardiology review was normal. She was counselled regarding the future likelihood of premature ovarian insufficiency (POI), the role of oocyte retrieval (OR) and cryopreservation, and pregnancy outcomes in TS patients. NHS exceptional funding for OR was declined. At 20, she requested oocyte cryopreservation. Her AMH was 52 pmol/L. Transvaginal ultrasound scan demonstrated PCO with an antral follicle count of 17 on her left (ovarian size 28x19x19mm) and >20 on her right (29x22x18mm). She was commenced on metformin. Stimulation was with 100 i.u. of follitropin alfa and ganirelix acetate (0.25mg on day 5). On day 12 the endometrial thickness was 13.3mm, with several follicles >18mm. On day 14, 14 oocytes at metaphase II were retrieved and cryopreserved. At 21, she underwent a second OR with 100 i.u. of follitropin alfa and cetrorelix (0.25mg on day 5). On day 17, 5 oocytes at metaphase II were collected and cryopreserved. To date none of the oocytes have been fertilised nor resulting embryos implanted. This suggests, that performing an ultrasound and checking AMH levels should be considered in patients with TS who have undergone spontaneous puberty to allow for appropriate fertility preservation counselling.

P109 A comparison of origio gamete handling products with sage and hartmann's solution

Turkay Gizem; Rattos Annabel; Salim Rehan; Jansa Perez Marta

Wolfson Fertility Centre

This study aimed to compare ORIGIO Sperm Wash Media (SWM) with SAGE Quinn's SWM for sperm preparation and ORIGIO Flush media (FM) (with heparin) with Hartmann's Solution (HS) (without heparin) for flushing. This data analysis included 90 cycles for the SWM comparison between March-May 2019 and 56 cycles for the FM comparison during July 2019. Data was divided into ORIGIO media products versus SAGE/HS. Gamete yield and fertilisation rates (FR) 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. Gamete handling media can play an important role in optimising gamete yield and quality, supporting the subsequent embryo development and therefore affecting cycle outcomes. Determining the optimal SWM and FM will allow improving gamete yield and FR. The average sperm yield in the ORIGIO group was slightly higher compared to the SAGE group (84.09% vs. 78.63% and 13.4M/ml vs. 8.20M/ml, $n=45$), although not statistically significant. When comparing IVF and ICSI cycles the average final sperm concentration in the ICSI ORIGIO group was significantly higher than the SAGE group (11.08M/ml vs. 4.01M/ml, $P=0.03$). This was not observed in the IVF group, which may be due to differences in group sizes (IVF $n=15$, ICSI $n=31$). However, the FR was comparable across all groups. For FM, egg yield, maturity and FR between ORIGIO and HS were comparable. The clotting rate in the ORIGIO group was slightly lower (3.57% vs. 10.71%) and the implantation rate (IR) was slightly higher (85.71% vs. 66.67%), however not statistically significant. ORIGIO SWM has been shown to result in a higher sperm yield, especially in the ICSI group. The trend towards the decreased clotting, higher FR and increased IR need to be confirmed with further data.

P111 Phospholipase C zeta profiles are indicative of optimal sperm parameters in patients undergoing fertility treatment

Kashir Junaid¹; Mistry Bhavesh²; BuSaleh Lujain³; AbuDawas Reema³; Nomikos Michail⁴; Aylan Ahmed²; Abu-Dawud Raed²; AlYacoub Nadya²; AlHassan Saad²; Lai F.Anthony⁴; Assiri Abdullah²; Coskun Serdar²

¹Cardiff University, Alfaisal University, King Faisal Specialist Hospital and Research Centre; ²King Faisal Specialist Hospital and Research Centre; ³Alfaisal University; ⁴Qatar University

Background: Oocyte activation is driven by intracellular calcium (Ca^{2+}) oscillations induced by sperm-specific phospholipase C zeta (PLC ζ). Sperm PLC ζ abrogation underlies cases of oocyte activation deficiency in humans. However, there is a lack of consensus regarding PLC ζ characterisation within human sperm due to concerns

surrounding antibody specificity and methodology. Furthermore, PLC ζ investigations have been limited to severe cases of male infertility, while PLC ζ profiles have yet to be associated with general sperm viability. Herein, we perform the first systematic effort to examine PLC ζ within a general population of males exhibiting a wide range of sperm parameters.

Methods: A total of 65 patients were recruited following informed written consent. Sperm concentration, motility, morphology, and semen volume were recorded and samples subject to density gradient washing. Sperm were subject to immunofluorescence and immunoblotting using two distinct antibodies with a high degree of specificity against PLC ζ .

Results: We identified PLC ζ at the equatorial and acrosomal+equatorial regions of the sperm head, alongside a novel dispersed pattern of PLC ζ , while also confirming the veracity of PLC ζ tail and mid-piece localisation in human sperm. Acrosomal+equatorial PLC ζ was most physiologically relevant to successful oocyte activation, while dispersed localisations correlated to sperm with sub-optimal parameters of sperm health. Although total levels of PLC ζ exhibited significant correlations with sperm parameters, the nature of this association requires further elucidation. Finally, PLC ζ variance in total levels corresponded to reduced sperm health, potentially underlying cases of increased fertilisation failure associated with cases of male subfertility and increasing male age.

Conclusions: Collectively, our results indicate for the first time that PLC ζ potentially represents a powerful target to measure sperm health when used with specific tools and protocols. We also suggest that our described methodology may assist in the similar utilisation and application of other sperm proteins in a clinical setting.

P112 Antioxidant effects of calligonum extract on ovarian tissue of PCO model: An experimental study

Movahedin Mansoureh; Tahmasebi Fatemeh; Mazaheri Zohreh

Tarbiat Modares University

Background: Polycystic ovary syndrome (PCO) is one of the most common reasons for infertility. Calligonum as a plant possess some of the important antioxidants that can decrease oxidative stress. The aim of this study was to study the effects of treatment with Calligonum as an antioxidant on ovary tissue of a PCO mouse model. MATERIALS AND

Methods: Thirty female NMRI mice were divided into three groups (n=10/each): control, PCO, and Calligonum. We induced PCO model with single dose of Estradiol valerate (40 mg/kg). Then Calligonum (20 mg/kg) was intraperitoneally injected weekly for two months. The level of oxidative stress and total antioxidant capacity was assessed in the ovarian tissue by flow cytometry and fluorescence recovery after photobleaching, respectively, and the histological study was conducted by the morphometric method and embryo development with in vitro fertilization.

Results: The obtained results showed that estradiol valerate was able to increase oxidative stress within the ovary and causes ovarian cysts after two months. The cyst formation was decreased in Calligonum group compared to PCO group (p=0.001). The percentage of pre-antral and antral follicles significantly decreased in Calligonum group compared to PCO group (p=0.001). The oxidative stress decreased in Calligonum group significantly compared to PCO group (p=0.001). Calligonum can significantly increase the total antioxidant capacity of ovarian tissue (p=0.001) as well as the percentage of in vitro fertilization compared to the PCO group.

Conclusion: Calligonum could decrease ovary cyst in PCO model, and improve in vitro fertilization rate. Also, Calligonum extract as an antioxidant could decrease oxidative stress in PCO model. KEYWORDS: Antioxidant; Calligonum; In vitro fertilization; Mouse; Polycystic ovary syndrome

P113 The impact of paternal diet on fetal growth and placental renin-angiotensin system gene expression

Khoshkerdar Afsaneh; Morgan Hannah; Watkins Adam

University of Nottingham

There is growing evidence linking paternal diet with fetal growth and adult cardio-metabolic disease risk. However, the underlying mechanisms are still poorly defined. The aim of this study was to assess the impact of paternal diet on fetal growth and the placental expression of multiple renin-angiotensin system (RAS) genes, known to be involved in placental blood flow and therefore fetal growth. 7-week old male C57BL/6J were fed either normal protein (NPD; 18% casein), low protein (LPD; 9% casein), a low protein diet supplemented with methyl-donors (MD-LPD), a western diet (WD, 24% fat) or a western diet supplemented with methyl donors (MD-WD) for a minimum of 8 weeks. Males were mated with virgin 8-12 week old female C57BL/6J mice, which were maintained on standard rodent chow. Pregnancy was allowed to progress to embryonic day 17.5 before the dams were euthanized and the fetal and placental tissues weighed and collected. Placentas were snap frozen for analysis of RAS genes expression using RT-qPCR. Paternal diet did not significantly alter mean fetal weight. However, placentas derived from MD-LPD males weighed significantly more when compared to WD placentas. Fetal-placental ratio in the WD group was significantly increased when compared to the MD-WD group. Conversely, the fetal-placental ratio in the MD-LPD group was significantly decreased when compared to LPD offspring. Analysis of placental RAS genes expression revealed significantly decreased expression of ACE, ACE2, Ren1 and Agtr1a in placentas from WD and MD-WD males when compared to NPD and LPD males. The current study provides evidence that sub-optimal paternal diet impacts fetal growth mediated potentially via gene regulating placental blood flow. Further investigations are required to determine how paternal diet influences placental function as well as their impact on maternal cardiovascular health during gestation.

P114 The classification and characterisation of human corpus luteum development and demise

Nazari Ahmed; Rice Suman¹

¹St George's University London

Background: The corpus luteum (CL) plays an integral role in hormonally dictated menstrual cycle in women¹. Formed from the remnants of the Graafian follicle it undergoes luteinisation of its constituent granulosa (GC) and theca cells (TC), in preparation for steroidogenesis. The life cycle of CL in a non-pregnant woman is 14 days beyond which point luteolysis occurs. Within a pregnant cycle the CL is rescued due to the expression of human chorionic gonadotrophin (hCG) acting on luteinising hormone receptors (LHR) ². Initiation of luteolysis is unknown in humans; whilst animal studies conducted on primates present luteolytic factors are responsible for luteolysis.

Aims: This study aimed to establish a classification system for the life cycle of the CL into early, mid and late luteal phases; whilst exploring the expression of oestrogen receptors ER α , ER β , progesterone receptors (PR) and Luteinising Hormone receptor (LHR) and their correlation with luteolysis.

Method: H&E and trichrome staining methods were used to identify physical characteristics within archived, fixed human corpora lutea tissue samples, in order to characterise them into early, mid and late luteal phase categories (n=3 at each stage). Immunohistochemistry was then used to determine the expression of ER α , ER β , PR and LHR and overall staining intensity determined.

Results and conclusion: We successfully established a classification system for various stages of the CL life cycle that correlated with established trends in expression of LHR and PR^{2,3}. All receptors showed high intensity staining at the mid luteal phase with a decreasing trend towards the late luteal phase, with the exception of LHR which maintained expression throughout. ER α was not detected in the early luteal phase and also had the lowest expression level throughout the other two stages; supporting the hypothesis that ER α :ER β may have significant role in bringing about luteolysis under the regulation of PGF 2α ⁴.

References 1. Luigi Devoto, Ariel Fuentes, Paulina Kohen, Pablo Céspedes, Alberto Palomino, Ricardo Pommer, Alex Muñoz, Jerome F. Strauss III "The human corpus luteum: life cycle and function in natural cycles" (2009) *Fertil. Steril.* 92:1067-79 2. W. Colin Duncan, Eva Gay and Jacqueline A Maybin "The effect of human chorionic gonadotrophin on the expression of progesterone receptors in human luteal cells in vivo and in vitro" (2005) *Reproduction* 130: 83-93 3. Y. Yung, S. Aviel-Ronen, E. Maman, N. Rubinstein, C. Avivi, R. Orvieto, A. Hourvitz "Localization of luteinizing hormone receptor protein in the human ovary" (2014) *Mol. Human Reprod.* 20: 844-849 4. Diaz, J. F., Wiltbank, C. M. "Acquisition of Luteolytic Capacity: Changes in Prostaglandin F 2α Regulation of Steroid Hormone Receptors and Estradiol Biosynthesis in Pig Corpora Lutea" (2004) *Biol. Reprod.* 70:1333-1339

P115 The role of the APC/C inhibitor Emi2 in the regulation of Meiosis I in mouse oocytes

Marangos Petros; Niaka Konstantina; Gonzalez Malagon Sandra; Stamatiou Konstantinos;
Bellou Irini; Papadopoulou Eleni; Labrianidou Andromahi

University of Ioannina

Mammalian oocytes enter meiosis before birth, where they remain arrested at G2/Prophase. Therefore, oocytes may lie dormant in the ovary for many years (>40 in humans) until they receive the stimulus to enter the first meiotic M-phase (MI) [1]. MI progression is regulated by the Spindle Assembly Checkpoint (SAC). SAC operates physiologically to inhibit the APC/C so that anaphase onset is delayed until all chromosomes are under tension on the M-phase spindle [2]. After the first meiotic division, subsequently the oocyte enters the second meiotic M-phase and arrests at Metaphase II (MII) awaiting fertilization. MII arrest is maintained through the action of the APC/C inhibitor, Early Mitotic Inhibitor 2 (Emi2) [3]. We are investigating whether Emi2 functions as a general surveillance factor that may arrest oocytes in M-phase when the spindle or chromosomes are damaged. Firstly, we have established that Emi2 is enabling an MII-related arrest state in MI. In addition, we have found that Emi2 depleted oocytes, under conditions of spindle damage, fail to launch an MI arrest response to chromosomal instability. This implies that Emi2 participates, alongside the SAC, in an M-phase checkpoint that safeguards the oocytes from inappropriate cell division following disruptions in spindle formation and chromosome alignment. Furthermore, in somatic cells, through FACS analysis and immunostaining protocols we found that Emi2 may potentially act as a cytostatic factor and its expression leads to cell cycle delay or arrest at G2/M.

1. Mehlmann, L.M. (2005). Stops and starts in mammalian oocytes: recent advances in understanding the regulation of meiotic arrest and oocyte maturation. *Reproduction*. 130, 791-799. 2. Musacchio, A. and Salmon, E.D. (2007). The spindle-assembly checkpoint in space and time. *Nat. Rev. Mol. Cell Biol.* 8, 379-393. 3. Madgwick, S., Hansen, D.V., Levasseur, M., Jackson, P.K., and Jones, K.T. (2006). Mouse Emi2 is required to enter meiosis II by reestablishing cyclin B1 during interkinesis. *J. Cell Biol.* 174, 791-801.

P116 Recovery of vaginal microbiota after standard treatment for bacterial vaginosis infection: an observational study

Lehtoranta Liisa¹; Hibberd Ashley¹; Reimari Jenni¹; Junnila Jouni²; Yeung Nicolas¹; Maukonen Johanna¹; Crawford Gordon³; Ouwehand Arthur¹

¹DuPont Nutrition & Biosciences; ²Pharma Ltd; ³CPS Research

Background: Dysbiosis of vaginal microbiota and Bacterial Vaginosis (BV) has been linked to negative reproductive health, e.g. decreased fertility and adverse pregnancy outcomes. Although BV can be treated with antibiotics, the recurrence rates remain significant. Understanding changes in vaginal microbiota composition in BV during and after antibiotic treatment would aid in making more accurate decisions on the treatment regimen and avoid unnecessary use of antibiotics. The purpose of the present study was to investigate vaginal microbiota and Nugent score changes after 5-day metronidazole treatment of BV and compare the results with healthy women.

Methods: This observational study was conducted in 30 healthy women (Group 1) and 30 women with BV (Group 2). Vaginal microbiota changes were assessed by Nugent score and by sequencing the V4 region of 16S ribosomal gene in swabs collected at baseline Day 1, as well as at Day 8 and 15, after completion of antibiotic treatment by women with BV.

Results: At Day 1, prior metronidazole treatment, significantly more women in Group 2 had elevated Nugent scores as compared to Group 1. The most abundant species in Group 1 were *Lactobacillus iners* (37.5%), *Lactobacillus crispatus/acidophilus* (19.2%), and *Gardnerella vaginalis* (9.6%), whereas in Group 2, the most abundant species were *L. iners* (25.8%), *Prevotella timonensis/bivia* (18.0%), and *G. vaginalis* (14.6%). After treatment on Days 8 and 15, Nugent scores were not different between the two groups. The microbiota was dominated by *L. iners*, and on Day 8 its abundance was significantly higher in Group 2 compared with Group 1 (67.8% vs. 37.5%, $P=0.049$). On Day 15, the relative abundance of microbial taxa was similar between the groups.

Conclusions: Metronidazole treatment of women with symptoms of BV shifted the vaginal microbiota to resemble that of healthy women after completion of antibiotic treatment.

P117 Sexually transmitted infections (STI) screening in fertility setting: A regional survey

Dreyer Frieda-Elsje; Ogbonmwan Daisy; Hussey Jane; Gudipati Madhavi

Sunderland Royal Infirmary

Background: NICE Guidance on fertility was updated in September 2017, however the guidance on chlamydia and other viral STI screening remains essentially unchanged from 2004. BFS Policy and practice document in 2010 on chlamydia screening has been broad and general. We aimed to survey the practice of STI screening in various fertility units in North east UK.

Methods: A practice questionnaire was sent to eight fertility units across the North East.

Results: Seven units out of the eight, including three tertiary care centres out of the eight completed the questionnaire. Reassuringly, all the units screened women for chlamydia before tubal testing as per the NICE guidance. Five of the seven offered this routinely as baseline test. The mode of screening however varied widely from urine [2 units] vaginal swabs [4 units] cervical swabs [2 units] and serology [2 units]. Obviously few units used more than one specimen. Two units screened for chlamydia antibodies to decide on the appropriate tubal test based on the titres, but did not follow up positive serology results with a test to rule out current Chlamydia infection. Three units gave prophylactic antibiotics [one prior to laparoscopy and the others for positive chlamydia antibodies] which varied from Azithromycin alone or combined or with metronidazole. Two units tested men with leucospermia for chlamydia by sing urine PCR. Four units screened couples for HIV, Hepatitis B and C prior to IUI / IVF as per NICE, but few units tested couples also for gonorrhoea, mycoplasma, trichomonas and syphilis.

Conclusions: Regional survey of fertility services has shown a wide variation in screening for STIs. This along with rising STI prevalence, newer sensitive and inexpensive STI screening methods available, it is time to re-evaluate and update the National guidance on STI screening in fertility setting to standardise the practice.

1. Fertility problems: assessment and treatment; Clinical guideline 156; September 2017; www.nice.org.uk
2. BASHH Guidelines; www.bashh.org

P118 Evaluation of embryo utilisation and pregnancy outcomes for three recombinant gonadotropins when compared with human menopausal gonadotropin

Fernandez-Ponce Alejandro; Yell Dawn; Drakeley Andrew; Gregoire Rachel

Hewitt Fertility Centre

There is a lack of robust clinical evidence to determine whether there are significant differences between urinary and recombinant gonadotropins in terms of embryo utilisation and pregnancy outcomes. However, there is some evidence indicating that urinary gonadotropins slightly increase pregnancy outcomes in advanced maternal age (AMA). The aim of this study was to evaluate the effectiveness of three recombinant gonadotropins against a human menopausal gonadotropin (hMG) currently in use at the Hewitt Fertility Centre (HFC) comparing embryo utilisation and pregnancy outcomes. This study included data comparing the fate of 6768 embryos from 941 cycles between July 2018-19, and analysing pregnancy outcomes. Patients were allocated into four groups; follitropin alpha 1 (389), follitropin alpha 2 (212), follitropin delta (43) and hMG (297). After comparison of the four groups, the findings show no significant differences in embryo utilisation rate (40.7% vs 43.1% vs 44.9% vs 44.2%) and positive pregnancy test (47.7% vs 36.6% vs 51.6% vs 44.4%), clinical pregnancy (35% vs 23% vs 45.2 vs 37.1), implantation (31.9% vs 18.7% vs 41% vs 29%) and pregnancy loss (12.7% vs 18.2% vs 14.3% vs 11.3%) rates. Parameters were also compared for the AMA group (>38 years) (patient numbers were 89, 54, 10, 88 respectively). No significant differences were found in embryo utilisation rate (36.8% vs 40.7% vs 42.4% vs 45.1%) and positive pregnancy test (25.5% vs 32.5% vs 37.5% vs 33.3%), clinical pregnancy (20% vs 17.5% vs 25% vs 20.6), implantation (17.6% vs 13.5% vs 30.8% vs 25.4%) and pregnancy loss (9.1% vs 22.2% vs 33.3% vs 23.5%) rates. The four gonadotropins have been shown to be suitable for clinical use. Parameters such as live birth rates and cost effectiveness of each gonadotropin now need to be considered.

P119 Transporting vitrified donor eggs does not affect success: a review of 220 cycles

Thomson Andrew¹; Palmer Giles¹; Wen Lihua²; Wang Jinjun³; Linara-Demakakou Elena³; Sprober Peter¹; Meredith Melanie¹; Thackare Hemlata¹; Macklon Nick³; Ahuja Kamal³

¹London Women's Clinic Wales and Bristol; ²London Women's Clinic Darlington; ³London Women's Clinic

Egg freezing is the fastest growing trend in assisted reproductive technology (ART). In the UK, 1,462 cycles were performed in 2017 compared to 410 in 2012 (HFEA, 2018). This rise reflects more effective technologies and the increase in frozen donor egg programmes. Since 2014, London Women's Clinic (LWC) has operated a large donor egg freezing programme in conjunction with the London Egg Bank (LEB). Over 100 donors are listed on the LEB catalogue where recipients at any LWC clinic can purchase from with no waiting time. Oocytes are transported to the required centre. Since it has been suggested that vitrified oocyte transport may be harmful (Sansinena et al, 2018). This study aimed to assess, the impact of transport on clinical outcomes. Method: Retrospective analysis of 220 transport cycles performed between Jan 2016 and June 2019. 141 were performed at LWC Cardiff, 79 at LWC Darlington and compared against 570 cycles frozen and thawed at LWC Harley Street. The primary outcome was clinical pregnancy rate. Results: 200 of the 220 transport cycles resulted in embryo transfer and 83 in clinical pregnancies (41.5% per ET), compared to 46.6% in Harley Street. By 2018, LWC Cardiff achieved 55.3% (21/38) and LWC Darlington 61.5% (16/26) clinical pregnancy rates using transported oocytes. Neither clinical pregnancy rate, thaw survival fertilisation rates were significantly different between centres ($p < 0.05$). Of the 20 abandoned transport cycles, 10 were due to no blastocyst formation, 4 for failed fertilisation, 3 for failed thaw (all frozen in 2016) and 3 due to medical reasons. Conclusion: This study demonstrates excellent outcomes can be achieved using transported donor eggs and transport need not impact on success. The development of this programme has allowed recipients access to far more donors, largely eliminating waiting lists. Centralised frozen donor egg banks are the future.

P120 Classification of late development stages of embryo with artificial intelligence algorithms

Mendizabal-Ruiz Gerardo¹; Chavez-Badiola Alejandro²; Flores-Saiffe Farias Adolfo²; Drakeley Andrew³

¹Universidad de Guadalajara; ²New Hope Fertility Center; ³Hewitt Centre for Reproductive Medicine

Background: Evidence suggests that the transfer of blastocyst in their hatching or hatched stages yields to better pregnancy rates in comparison with the transfer of blastocyst in the expanding phase. Therefore, success in assisted reproduction may be improved if the selection is based on hatching status. With the increase of time-lapse incubators in the clinics, it is relevant to count with algorithms that can automatically determine the stage of an incubated embryo.

Objective: To create a deep learning model capable of automatically determine the stage of a blastocyst from a single microscopy image.

Methods: We retrospectively employed a database of 1,025 blastocyst micrographs obtained from three different clinics using five different laboratory microscope settings. All images depicted blastocyst in either of three phases: expanding, hatching or hatched. An expert embryologist classified each embryo based depicted in the photographs. A convolutional neural network architecture based on inception modules was custom developed (see Figure 1) and trained using 70% of the database employing data augmentation. The remaining 30% of the images were used for validation of the trained network.

Results: The proposed network was able to classify the unseen blastocyst images with performance up to 90% of accuracy after 500 training epochs (see Figure 2).

Discussion: Despite the variability of the micrographs due to different settings, the proposed deep learning algorithm was successful in performing the classification of the test dataset. This type of algorithms that perform classification tasks may help to pave the way to the development of fully automatic incubators that are able to determine the best moment to perform the transfer of the embryos.

P121 Can artificial intelligence boost chances of success in IVF?

Biswas Shivhare Sourima¹; Srikantharajah Arasaratnam²; Carroll Michael³; Homburg Roy¹

¹Homerton Fertility Centre; ²Homerton University Hospital; ³Manchester Metropolitan University

Background: Globally, 2% of women experience primary infertility resulting in an increased use of assisted reproductive technologies (ART). However, live birth (LB) rates from ART remained stagnant over the last decade due to limitations in understanding of in vitro human embryo development and implantation potential of the endometrium. Additionally, the choice of embryo(s) to transfer depended primarily on morphology (CMG), which remains rate limiting. Interestingly, time-lapse imaging (TLI) incubators such as an EmbryoScope (Vitrolife) has allowed continuous monitoring of embryo development, utilising morphological and kinetic parameters (KIDScore), without exposure to environmental stress. Multiple studies have shown improvement in success rates using Embryoscope; although results remain varied. Furthermore, EmbryoScope culture consists of two elements; continuous undisturbed culture and monitoring of morphokinetic parameters, which no one study has been able to clearly dissect in conjunction with LB.

Aim: Does the use of EmbryoScope have an impact on LB from either undisturbed culture or the use of KIDScore for embryo selection for transfer?

Methods: This is a retrospective study of 490 single embryo transfers [MINC, (COOK) and EmbryoScope]. The study compared the mode of embryo culture and selection method for transfer to LB.

Results: The study showed (i) LB was significantly higher for a D5 blastocyst transfer (Chi-squared $p < 0.0001$), (ii) a trend towards higher LB from EmbryoScope (Fisher's Exact test $p = 0.08$), (iii) CMG differed from KIDScore (D5 ET $\kappa = 0.11$) such that CMG better corresponded to LB and (iv) KIDScore algorithm may be personalised to correspond to LB for individual fertility centres.

Conclusions: This is a retrospective single centre study, which does not differentiate between patient age, ethnic background, stimulation protocol or treatment type; therefore, results may not be extrapolated. However, it sets the pathway for a prospective randomised control trial, which would determine any clinical benefit of utilising an EmbryoScope within IVF.

P122 Fertility predictor: The first AI-driven empowering tool providing patients with realistic expectations associated with different types of treatment

Barkaoui Samya; Jacques Celine; Kotrotsou Mara; Beaumont Lucy; Louzada Julio; Noublanche Caroline; Hickman Cristina

Apricity

Objectives: To develop an online tool to help patients predict their chance of live birth (LB) after IVF treatment, according to a patient's specific characteristics.

Methods: Based on a population-based cohort study with 162 cohorts of patients analysed from the most recent 500,000 cycles from the HFEA register, from 2010 to 2016. Prediction of cumulative LB after three cycles using 6 machine learning algorithms.

Results: Key predictors of LB (in order of importance), were female age (18-34:43%, 35-37:22%, 38-39:15%, 40-42:14%, 45-50:2.2%), date of embryo transfer, total number of embryos created, number of cumulative cycles (1st:41.2%, 2nd:25.2%, 3rd:14.5%, 4th:8.1%, 5th:4.5%, 6th:2.6%, >6th cycle: 3.8%); embryos transferred (Importance=0.4, 0.24, 0.08, 0.06, 0.05 respectively). Number of fresh eggs collected and type of treatment (FET: 22.2%, IVF:36.6%, ICSI: 41.2%) approached significance (Importance=0.04), whilst causes and duration of infertility, and stimulation were not significant determinants of LB (importance < 0.05). A 43 year old with unexplained infertility has a 16% chance of LB after three cumulative fresh medicated IVF cycles (6, 11, 16% after 1st, 2nd, 3rd cycle respectively), but a higher ($p < 0.001$) chance after frozen cycles (10, 19, 26%), and a higher ($p < 0.001$) chance after one egg donation cycle (39%). Using donor sperm increased the chance of LB from 6 to 9%.

Conclusion: Fertility Predictor makes the most recent HFEA dataset accessible to patients, in a way that can be readily interpreted and visualised to assist the patient in making decisions based on realistic expectations regarding their specific treatment options. This is the first time that AI technology is used to build a powerful and empowering decision tool for patients.

P123 Suboptimal quality embryos reduce the implantation potential of good quality embryos

Whitten Bryony; Gregoire Rachel

Liverpool Women's Hospital NHS Foundation Trust

Background: There is increasing evidence to suggest that decidualised endometrial stromal cells can differentiate between embryos of differing quality, with developmentally impaired embryos potentially provoking an endoplasmic stress response that results in implantation failure. The aim was to investigate whether simultaneously replacing a good quality embryo with one of poorer quality impacts upon endometrial receptivity and negatively affects implantation, in comparison to replacing a good quality embryo alone.

Methods: A retrospective analysis of patients undergoing a fresh embryo transfer between March 2014-April 2019 was performed (n=3836). Positive pregnancy test, biochemical pregnancy, clinical pregnancy (CP) and implantation rates (IR) were compared in patients having SET with a good quality embryo (G) (n=3465), compared to DET of a good combined with a poor (G&P) (n=156) or average embryo (G&A) (n=215). Quality was scored using the ACE Embryo Grading Scheme (2010 & 2017) and categories of good, average and poor quality were devised.

Results: Positive pregnancy test, CP and IR were significantly higher in patients in the G group compared to G&P ($p<0.000004$; $p=0.000126$; $p<0.000001$) or G&A ($p=0.000265$; $p=0.001679$; $p<0.000001$). An increased rate of biochemical pregnancy was observed in both G&P and G&A compared to the G group, but was not significant at $p<0.05$ ($p=0.066$ and $p=0.339$, respectively). Average age within each group was 35 years and there was no significant difference in results across individual ages (<35; 35-37; 38-39; 40+).

Conclusions: A significant reduction in implantation rate, without a significant increase in biochemical loss, indicates that the presence of a suboptimal embryo prevents implantation of good quality embryos. This study therefore suggests that replacing a second embryo of suboptimal quality with a good quality embryo does trigger an endometrial response leading to implantation failure. These data support eSET in circumstances whereby only a single good quality embryo is available for transfer.

P124 HRT in autologous frozen embryo transfer cycles is associated with increased biochemical pregnancy rate

Hamilton Tracey¹; Dieu Ly Bui²; Noble Clare¹; Samet Rebecca¹; Lyon Jennifer¹; Gaudoin Marco¹; Fleming Richard¹

¹Glasgow Centre for Reproductive Medicine Fertility; ²University of Glasgow

Purpose: To determine differences in outcomes in natural vs. medicated frozen embryo transfer (FET) treatment cycles.

Methods: Clinical pregnancy rates (CPR) and pregnancy loss rates from FET cycles were determined from a single centre from January 2017 to July 2019 inclusive. 498 women (541 cycles) had a natural cycle-FET (NC-FET) and 284 women (327 cycles) used HRT. Patients were subdivided into NC-FET or HRT-FET using (a) their own (autologous) eggs or (b) donor eggs. HRT-FET cycles were further subdivided by method of control: GnRH-antagonist or GnRH-agonist.

Results: In embryos created from donor eggs, there was no difference in outcomes in GnRH-antagonist (N = 78) or GnRH-agonist (N = 97) cycles. In women using donor eggs there was no difference whether NC-FET (N = 31) or HRT (N = 175) was employed, although there was a trend to lower CPR (37% vs. 54%), and higher biochemical pregnancy rates (18% vs. 8%) in the HRT group. In the autologous egg group: 276 women (479 cycles) underwent NC-FET (Gp-Own) and 77 women (127 cycles) underwent HRT-FET (Gp-HRT). There was no difference in age but Gp-HRT had higher AMH and BMI ($P < 0.001$). Pregnancy rates were similar (58% and 61% respectively) but biochemical pregnancy rates were higher in Gp-HRT (26% vs. 13%, $P = 0.001$).

Conclusions: Pregnancy loss rates may be higher in HRT cycles (comparing groups when embryos were created from donor eggs) but in women using their own eggs, the biochemical pregnancy rate was twice as high in the HRT group. These women had higher BMI and AMH so they were more likely to have polycystic ovary syndrome, necessitating an

HRT-FET cycle in the first place, and further work is required to determine if it is the obesity, the PCOS or the HRT which contributes to the increased loss rate.

P125 Extracellular vesicles isolated from culture media conditioned by individually cultured bovine embryos as a potential embryo quality marker

Dissanayake Keerthie¹; Nõmm Monika²; Lättikivi Freddy³; Ressaissi Yosra⁴; Godakumara Kasun³; Lavrits Arina³; Balcha Getnet³; Viil Janeli³; Bæk Rikke⁵; Jørgensen Malene Møller⁵; Bhattacharjee Sourav⁶; Andronowska Aneta⁷; Salumets Andres⁸; Jaakma Ülle⁹; Fazeli Alireza¹⁰

¹University of Tartu, Estonia; ²University of Life Sciences, Estonia; ³Institute of Biomedicine and Translational Medicine, University of Tartu, Tartu, Estonia; ⁴Faculty of Veterinary Medicine, University of Teramo, Italy; ⁵Department of Clinical Immunology, Aalborg University Hospital, Denmark; ⁶School of Veterinary Medicine, University College Dublin, Ireland; ⁷Institute of Animal Reproduction and Food Research, Polish Academy of Sciences, Poland; ⁸Department of Obstetrics and Gynecology, Institute of Clinical Medicine, University of Tartu, Tartu, Estonia; ⁹Chair of Animal Breeding and Biotechnology, Estonian University of Life Sciences, Tartu, Estonia; ¹⁰Department of Oncology and Metabolism, University of Sheffield, United Kingdom

Background: Extracellular vesicles (EVs) are membrane-bound biological nanoparticles (NPs). We aimed to isolate and characterize EVs from media conditioned by individually cultured preimplantation bovine embryos and to assess their relationship with embryo quality.

Methods: Embryos were produced as previously described (1). Presumptive zygotes were in-vitro cultured individually in 60 µl droplets of culture media and fifty microlitres of media were collected from the droplets either on day 2, 5 or 8 post-fertilization. After sampling, the embryo cultures were continued in the remaining media till day 8, and the embryo development was evaluated at day 2 (cleavage), day 5 (morula stage) and day 8 (blastocyst stage). NPs were isolated using qEVsingle® columns and characterized.

Results: Based on EV array (2), NPs isolated from embryo conditioned media were strongly positive for CD9 and CD81, weakly positive for CD63 and Alix among others, and showed cup-shaped EV structures in transmission electron microscopy and spherical EV shape in scanning electron microscopy, and hence regarded as EVs. However, NPs isolated from the control media were not positive for EV array or microscopical analysis and, hence regarded as NPs. Based on nanoparticle tracking analysis, at day 2, the mean concentration of EVs isolated from media conditioned by embryos that degenerated after cleaving ($8.25 \times 10^8/\text{ml}$) was higher compared to that from embryos prospectively developed to blastocysts ($5.86 \times 10^8/\text{ml}$) ($p < 0.05$). Moreover, at day 8, EVs isolated from media conditioned by degenerating embryos ($7.17 \times 10^8/\text{ml}$) was higher compared to that from blastocysts ($5.68 \times 10^8/\text{ml}$) ($p < 0.05$). Furthermore, at day 8, the mean diameter of EVs isolated from media conditioned by degenerating embryos (153.7 nm) was smaller than EVs from media conditioned by blastocysts (163.5 nm) ($p < 0.05$).

Conclusions: Individually cultured preimplantation bovine embryos secrete EVs in the culture media. Their concentration and size are influenced by embryo quality and may indicate their prospective development potential.

1. Nomm M, Porosk R, Pärn P, Kilk K, Soomets U, Koks S, et al. In vitro culture and non-invasive metabolic profiling of single bovine embryos. *Reprod Fertil Dev*. 2019

2. Jørgensen M, Bæk R, Pedersen S, Søndergaard EKL, Kristensen SR, Varming K. Extracellular Vesicle (EV) array: Microarray capturing of exosomes and other extracellular vesicles for multiplexed phenotyping. *J Extracell Vesicles*. 2013

P126 Does cell exclusion at the morula stage indicate embryo correction?

Quinn Chelsea; Thompson Karen; Brunt Amie

Leeds Fertility

Selection of the best embryo for transfer is vital for successful IVF outcomes. Time lapse imaging (TLI) has enhanced this decision making, with detailed information collected at each stage of development. It is important to understand the different development stages and how this impacts on final outcome. This study aims to analyse the impact of cell

exclusion at the morula stage on treatment outcomes. 524 embryos were retrospectively assessed at the morula stage using the Embryoscope. They were annotated from the fusion of the first two cells up to the first signs of cavitation. The morulae were analysed for the degree of complete or incomplete compaction, the percentage of excluded cells and fragmentation. Culture continued until day 5. 454/524 embryos formed complete morulae, 191 resulted in a live birth. 27 live births were achieved from 70 morulae graded as incomplete. There was no significant difference shown between the number of live births from complete or incomplete morulae ($P = 0.7248$). When observing percentage of excluded cells there was no significant difference in live birth rate ($P = 0.966$). However the quality of the blastocyst on day 5 was influenced by both factors. Completely compacted morulae showed a significantly higher rate of A/B graded blastocysts ($P = < 0.0001$) and a higher percentage of excluded cells showed a lower rate of A/B graded blastocysts ($P = < 0.00001$). Fragmentation showed a similar result with no impact on live birth rate ($P = 0.5$) but an increased percentage of fragmentation resulted in a significantly poorer quality blastocyst. The exclusion of blastomeres at the morula stage does have a significant impact on the quality of the day 5 blastocyst however this does not impact the live birth rate. This may suggest embryo correction occurs at the morula stage.

P127 The effect of prokineticin 1 on the angiogenesis in the endometrium during pregnancy

Goryszewska Ewelina; Baryla Monika; Kaczynski Piotr; Wacławik Agnieszka

Institute of Animal Reproduction and Food Research of Polish Academy of Sciences

Prokineticin 1 (PROK1), is also called as an endocrine gland-derived vascular endothelial growth factor (EG-VEGF). Studies in humans have shown that PROK1 plays a key role in regulating pregnancy-related processes such as angiogenesis, proliferation and regulation of the immune response. In the female endometrium, expression of PROK1 and its receptor (PROKR1) is increased during implantation and placenta development in the first trimester of pregnancy. The aim of the present study was to determine PROK1 effect on proliferation and capillary-like network formation of porcine endometrial endothelial cells.

Uteri were collected from gilts on day 15 ($n=10$) of the pregnancy. Endometrial tissue was digested by dispase and next by the collagenase. The cell mixture was treated by Dynabeads® CD31 Endothelial Cell according to manufacturers' protocol. To determine the PROK1 effect on proliferation, endothelial cells were seeded into a 96-well plate and cultured to around 40% confluence. Then, the cells were incubated 24 hours with 40 nM PROK1 in the presence or absence of 1 μ M PROK1 receptor antagonist (PC7). Cell proliferation was assessed by colorimetric method. To determine the PROK1 effect on the capillary-like network formation, endothelial cells were seeded on μ -Slide dishes and incubated 6 h with 40 nM PROK1 together/without 1 μ M PC7. Two-way ANOVA followed by Tukey post-tests was used to analyze obtained results.

Proliferation of endometrial endothelial cells was increased after incubation with PROK1 on the 15 day of pregnancy ($p<0.05$). This effect was abolished after using PC7. Moreover, PROK1 stimulated capillary-like network formation. A statistically significant increase was observed in 15 of the 20 parameters describing angiogenesis ($p<0.05$). Co-treatment with PC7 diminished the stimulating effect of PROK1.

Results of the present study indicate that PROK1 are important factors involved in the angiogenesis in the porcine endometrium during pregnancy.

P128 High prevalence of autosomal aneuploidies associated with early pregnancy loss (EPL) in the mare

Shilton Charlotte¹; Kahler Anne¹; Crabtree James²; Crowhurst James³; Davis Brian⁴; McGladdery Andrew⁵; Wathes Claire¹; Raudsepp Terje⁴; de Mestre Amanda¹

¹Royal Veterinary College; ²Equine Reproductive Services; ³Newmarket Equine Hospital; ⁴Texas A&M University; ⁵Rosssdales Veterinary Surgeons

Background: Survival of any species depends on the production of healthy offspring, with losses during pregnancy countering this requirement. Early pregnancy loss (EPL) occurs in 5-8% of confirmed equine pregnancies, with no

diagnosis made in over 80% of these cases. Whilst aneuploidy is implicated in a significant proportion of human spontaneous abortions, investigations into aneuploidy rates of other species remain limited. The objective of this study was to identify aneuploidies in equine EPL conceptuses.

Methods: EPL conceptus material and clinical histories were submitted from cases of pregnancy loss (14-65 days of gestation) between 2013 and 2018. Age-matched control conceptuses were obtained from manually terminated clinically normal pregnancies (CNP). Equal loads of isolated DNA from allantochorion and foetal tissues were hybridised to Axiom™ Equine Genotyping Array (Affymetrix, USA). DNA from healthy term chorioallantois and adult blood were also present on the array. Whole genome copy number was visualised using Integrative Genomics Viewer (IGV).

Results: Aneuploidy of at least one chromosome was detected in 12/55 EPLs (21.8%), compared with 0/10 CNP, 0/5 healthy term, and 0/5 healthy adult mares. Aneuploidies involved 10/32 chromosomes, representing both trisomies ($n=9/12$) and monosomies ($n=3/12$). Only 2 of the autosomal aneuploidy types have been previously reported in live-born equines (trisomy 30, and trisomy 23) with the remaining aneuploidy types being unique to this study. Maternal age did not significantly differ between aneuploidy and non-aneuploid EPL conceptuses (range 3-19, and 4-21 years, respectively).

Conclusions: We present the first evidence of aneuploidies in conceptuses from failed equine pregnancies offering the first step in identifying definitive genetic causes for these early losses. Aneuploidy is thus not human-specific and may offer answers to pregnancy loss in other domestic species.

P129 IVF/ICSI splits: the best of both worlds?

Shakur Farah; Murray Joanna; Chetty Maya

Edinburgh Fertility Centre

Objective: If there is uncertainty regarding optimal mode of insemination an IVF/ICSI split is sometimes offered. The aim of this study was to compare the outcomes of IVF and ICSI in split cycles. The primary outcome was to identify any differences in the fertilization rate and embryo development. The secondary outcome measure was clinical pregnancy rate.

Materials and methods: This was a retrospective study of the split IVF/ICSI cycles from January 2017 to December 2018. Data was collected from the embryology database and was analysed using GraphPad. 19 split IVF/ICSI cycles were identified. The oocytes were split into ICSI ($n=99$) and IVF ($n=136$). The median age of patients was 37 years. Semen parameters assessed according to WHO 2010 classification. The most common indication for the split was previous poor samples. One of the borderline samples on the day did not meet the criteria for IVF but prepared well.

Results: The fertilization rate was 155 out of 237 (65.4%). ICSI 66.7%, IVF 65.4% ($p=0.09$). There was no case of complete failed fertilization. There was no statistically significant difference in the number of good quality embryos on day 2, day 3 and day 5. Three patients had freeze all because of hyperstimulation. Six had day 3 transfers, 10 had blastocyst transfers. Of the 16 embryo transfers nine had ICSI and six had IVF embryos transferred. One patient had a double embryo transfer of one IVF and one ICSI embryo. The clinical pregnancy rate in the fresh cycles was 10/19 (53%); 6/9 for ICSI and 4/6 for IVF ($p=0.72$). The cumulative pregnancy rate including thaw cycles is 13/19 (68%).

Conclusion: According to our small study there is no advantage in doing ICSI when there is no clear indication to do so and so IVF/ICSI splits do not need to be offered.

P130 Obstetric outcomes of pregnancies after fertility treatment

Steshenko Alexander¹; Ketova Olena²; Liakhovska Tetyana²; Hanna Leila¹

¹Queen Elizabeth Hospital; ²City Maternity Hospital, Poltava, Ukraine

Background: It is estimated that infertility affects 1 in 6 couples. Advances in assisted reproductive technologies offer a realistic opportunity to men and women with fecundity issues to have a child of their own. However, when fertility treatment is successful and pregnancy is achieved, the women very often face further challenges such as increased risks of antepartum and intrapartum complications associated with using assisted conception technologies. The aim of this study was to find what obstetric outcomes are in women who became pregnant as a result of fertility treatment.

Methods: A cohort study with a retrospective review of medical records of women giving birth at a hospital in Ukraine over three years and a similar hospital in the UK over one year. The study included cases where women achieved pregnancy via fertility treatment such as ovulation induction, assisted conception with IUI, IVF, and ICSI. Outcomes of interest were rates of multiple pregnancies, pre-eclampsia, gestational diabetes, preterm births, induction of labour, mode of delivery.

Results: Hypothyroidism was diagnosed in 6.7-14.2% of women, 8.2% had gestational diabetes, and 1.5-1.7% had severe pre-eclampsia. Overall 11.2% of women gave birth at <37 weeks of gestation. Preterm deliveries were 45.5% in twin pregnancies and 8.1% in singletons. Induction of labour was conducted in 21.6% of cases. Vaginal delivery was achieved in 6.6% of women from Ukraine and 53% from the UK. The caesarean section rate was 93.4% in Ukraine and 47% in the UK. Twin pregnancies accounted for 3.3-8.2% of all cases. The highest rate of caesarean section was in women with twin pregnancies (73%-100%).

Conclusion: Women who conceived with fertility treatment have higher rates of hypothyroidism, preterm deliveries, twin pregnancies, and caesarean sections. The finding of the study can assist clinicians in counseling women on expected obstetric outcomes of pregnancies after fertility treatment.

P131 Exploring the tool kit: application of Quality Improvement (QI) principles founded from manufacturing within the IVF laboratory using the Model for Improvement (MFI) and lean

Woodland Emma; Monks Nicola; Barker Bridget; Howard Mark; Derry Sarah; Whale Lisa; Umranikar Aarti

Salisbury Fertility Centre

Background: QI principles conceived from the manufacturing industry were evaluated within an IVF clinic. MFI and lean can accelerate performance improvement within an organisation making it faster, better and more affordable. There are examples of successful application of these approaches within healthcare (1-3). Little has been published regarding application of MFI or lean within assisted conception. This study aimed to assess the usefulness of these QI principles within an IVF laboratory.

Methods: The MFI and lean were applied to identify areas for improvement within the laboratory processes. An area of focus was optimisation of culture conditions and changes for improvement were explored. Incubators were utilised differently. This was a prospective study. Many QI tools were used including Statistical-Process-Control charts (BaseLine© SAASoft). Measurements included incubator door openings and stability, practitioner steps, procedure timing, and standard clinical outcome data.

Results: Clinic staff engaged with the project which emphasised the importance of QI within the laboratory. Certain process measures indicated an improvement. The frequency of incubator door openings was reduced by 36%. The distance oocytes travelled within the laboratory was reduced by 22% and each culture dish was out approximately 15.5 seconds less during procedures. This resulted in a 9% reduction in the time that oocytes spent outside of optimum incubator culture conditions and removed approximately 9.5 steps taken by practitioners during procedures. The daily fluctuation of incubator O₂/CO₂ gas levels was significantly reduced. Other process measures showed no significant change (incubator temperature, fertilisation rates and embryo utilisation rates). Outcome measure of clinical pregnancy rate and implantation rate remained consistent.

Conclusions: This work resulted in improvement in the culture system workflow by refining processes, without impacting on clinical results. Team exploration of QI principles was a valuable learning experience encouraging a mindset of continuous QI and accelerated performance improvement within the IVF laboratory.

1) Silvester K. Learning with the Labs. JOIS. 2015;22:1-30.

2) Graban M. Lean hospitals: Improving quality, patient safety, and employee engagement. 3rd ed. Taylor & Francis group: New York; 2016.

3) Mazzocato P, Savage C, Brommels M, Aronsson H, Thor J. Lean thinking in healthcare: a realist review of the literature. *Qual Saf Health Care*. 2010;19:376-382.

P132 The impact of paternal diet on pre-implantation development and the metabolic sensor AMPK in murine embryos

Richardson Lauren; Nightingale Ian; Morgan Hannah L; Watkins Adam J

University of Nottingham

Background: The DOHaD hypothesis proposes early environmental influences can alter embryonic metabolism and development, impacting on the long-term health of the resultant offspring. While the impact of poor maternal diet on embryo development and long-term offspring health is well-established, the role of a father's diet is less well characterised.

Methods: Male C57BL/6 mice were fed either a normal protein diet (NPD; 18% protein), low-protein diet (LPD; 9% protein), Western diet (WD; 20% fat, 0.15% cholesterol), or a methyl donor supplemented LPD or WD (MD-LPD or MD-WD) for at least 8 weeks prior to mating with virgin chow diet fed C57BL/6 females. Oviducts of pregnant dams were flushed at embryonic day (E)1.5 and embryos were cultured to the blastocyst stage in KSOM, 5% CO₂, 37°C. Embryo development was assessed visually at regular intervals. Blastocysts expression of AMPK pathway-specific genes Trp53, Akt2, Adipor1, and Prkaa1 was assessed using qRT-PCR.

Results: There was no difference in embryo number between dietary groups, however there was a non-significant increase in fertilisation rates in MD-WD group. WD derived embryos had a lower proportion exceeding the developmental target at E2 ($p < 0.05$) and a higher proportion of embryos below developmental target at E2.5 ($p < 0.01$) compared to LPD. However, the number of blastocysts obtained at E3.5 did not differ across dietary groups. Analysis of gene expression revealed significantly higher Akt2 expression in LPD compared to WD derived embryos ($p < 0.05$) and higher expression of Prkaa1 in MD-WD derived embryos compared to NPD ($p < 0.01$).

Conclusions: Poor paternal diet disturbs pre-compaction development and blastocyst AMPK gene expression of the offspring, which can be further influenced by the addition of dietary supplements. Further studies are required to determine the long-term impact of these changes and whether they have clinically relevant implications for men.

P133 Reproduction post-myomectomy: guideline development

Brooks Charlotte; Webber Lisa

Imperial NHS Trust St Mary's Hospital

Reproduction post-myomectomy: Guideline development Background A myomectomy is a fertility-sparing procedure for women who either have symptoms related to their fibroids (menorrhagia/pressure symptoms) or where the fibroids are thought to be affecting their reproductive ability. This latter is often in the context of assisted reproduction. Currently there are no guidelines (RCOG/ACOG/NICE/ESHRE) on the management of reproduction (fertility or obstetric) post-myomectomy.

Objectives: What advice is being given to women post myomectomy regarding future pregnancies A review of the latest literature To formulate a guideline Improved consent pre-myomectomy of the potential future fertility issues.

Method: I surveyed Gynaecologists at a large London tertiary hospital these are some of the questions asked: What affects your decision to recommend caesarean section: number of fibroids; location; if endometrium breached; laparoscopic vs open? When would you advise them to conceive post op: 6/52; 3/12; 6/12; >1 yr; >2yr? If you deem the patient suitable for a vaginal delivery would you treat them like a VBAC in labour? I will also ask the same interactive online questionnaire to the audience.

Results: The majority would recommend a Caesarean section to women whose endometrial cavity was breached. The number of fibroids removed, the location and size of the fibroid was not relevant. Most would recommend waiting three or six months before trying to conceive again.

Discussion: Myomectomy is associated with uterine rupture in 0.4-0.9% of cases. It is unclear if time to next pregnancy following myomectomy influences this risk. Other potential risk factors are the size, number and location of the fibroids removed and whether or not the full thickness of the myometrium was breached.

Conclusion: A pragmatic, evidenced-based guideline would help manage the risks of pregnancy following myomectomy without potentially delaying unnecessarily the chance to conceive. This should include time to next pregnancy and obstetric management.

P134 Day 3 versus day 5 (blastocyst) transfer - Does it matter in poor responders?

Felix Nirmal; Datta Adiraja; Nargund Geeta; Campbell Stuart

Create Fertility

Background: The optimal management of poor responders remains a challenge despite a multitude of studies dedicated to improving outcomes in this group. There had been few studies looking into day 2 versus day 3 transfers in the poor responder's category with no conclusive answer.

Method: Retrospective analysis of sub fertile patients who had undergone frozen embryo transfers after natural/natural modified IVF/ICSI cycles from June 2016 for a period of 1 year across 2 fertility units using the same culture media and culture techniques. The study period spanned two distinct policy periods, where one of the sub unit actively performed day 3 transfers and the other did blastocyst transfers. 204 patients were included based on the Bologna criteria. 110 patients had day 3 transfer and 94 had blastocyst transfer. Main Outcomes measured were live birth rates per embryo transferred, clinical pregnancy rates and miscarriage rates. The t test was used to compare the continuous variables (means), and the chi-square test was used to compare categorical variables (percentages). P value of less than 0.05 was considered to be significant.

Results: The mean age, Anti Mullerian hormone, antral follicle count, Body mass Index and parity was similar in both the groups. There was no statistical difference in live birth rates (19 % vs. 15.9 %; p-NS) and clinical pregnancy rates (22.7% vs. 22.3 %, p- NS) among the groups. The Live birth rates and clinical pregnancy rates per embryo transferred was also comparable. There was no statistical difference in miscarriage rates between the groups.

Conclusion: Extended culture to blastocyst in poor responders may end up with higher higher cancellation rates, no embryos for transfer and freeze. We believe that this strategy may need further evaluation as this approach seems not to help in better selection of embryo in poor responders.

P135 Correlation of reproductive hormone profiles and semen quality

Petyim Somsin; Chukaew Ronnakorn; Thanaboonyawa Isarin; Prechapanic Japarath; Laokirkkiat Pitak; Choavaratana Rounsing

Department of Obstetrics and Gynaecology, Faculty of Medicine Siriraj Hospital

Objective: To evaluate the relationship between testosterone/estradiol ratio and semen quality.

Methods: Fresh semen samples were assessed for quality (concentration, motility, and morphology) and the serum hormone levels including follicle-stimulating hormone (FSH), luteinizing hormone (LH), Estradiol (E2), and Testosterone were measured. In total, 67 men (40.9%) were included in the abnormal semen group and subdivided into 3 groups according to their semen analysis results: asthenozoospermia, asthenooligozoospermia, and oligozoospermia.

Results: Serum FSH had a significant negative correlation with sperm concentration, motility, and normal morphology. Serum LH had a significant negative correlation with sperm motility and normal morphology. Serum FSH and LH levels were significantly higher for abnormal semen group compared with normal semen group. In asthenozoospermia group, estradiol and testosterone had a significant positive correlation with sperm concentration, but estradiol significantly negatively correlated with sperm motility. In oligozoospermia group, estradiol significantly negatively correlated with

normal sperm morphology. No significant correlation was found between semen and reproductive hormone parameters in asthenozoospermia groups. Moreover, no significant correlation was found between testosterone/estradiol ratio and semen quality in all groups.

Conclusion: There is no significant correlation between testosterone/estradiol ratio and semen quality in all groups. Future studies on the current topic are therefore suggested in order to establish a better understanding for the correlation of serum testosterone/estradiol ratio and semen quality.

P136 Does presence of a first trimester subchorionic haematoma during fresh IVF cycles impact on pregnancy outcomes?

Rouvali Angeliki; Balfoussia Danai; Stradiotto Lisa; Aziz Nader; Sarodey Geet; Rai Raj; Salim Rehan

Imperial College Healthcare NHS Trust

Background: The incidence of subchorionic haematoma (SCH) in fresh IVF cycles is not known, nor is its impact on treatment outcomes. We aimed to assess whether there is a correlation between presence of a SCH and live birth, timing of delivery and birthweight, and pregnancy loss.

Methods: This was a retrospective review of consecutive IVF/ICSI cycles between January 2012 and December 2018. Inclusion criteria were singleton intra-uterine pregnancy with fetal heartbeat at the six-week scan.

Results: 1446 cycles meeting the inclusion criteria were identified, of which 55 were excluded due to loss to follow-up. Of the remaining 1381 women, 53 (3.84%) had a subchorionic haematoma (SCH) at the six-week scan. There was no difference in the mean age between those without a SCH (Group 1) and those in whom a SCH was seen (Group 2): 33.48 ± 3.96 vs 33.76 ± 3.71 , $p = 0.602$.

The live birth rates were comparable in the two groups (89.5% vs 83%, $p = 0.133$). There was no difference in gestational age at delivery or birthweight [(38.39 ± 3.79 vs 38.37 ± 2.43 weeks, $p = 0.960$) and (3180.36 ± 617.56 vs. 3115.93 ± 736.87 grams, $p = 0.505$).

In those who had a pregnancy loss ($n = 134$), there was no association between the presence of a SCH and the trimester in which the pregnancy loss was diagnosed ($p = 0.164$).

Conclusion: Our study suggests that presence of a first trimester subchorionic haematoma during fresh IVF cycles does not have an adverse effect on treatment outcomes. Our findings are limited by potential reporter bias.

P137 Do women with a predicted poor ovarian response based on antral follicle count experience poor clinical outcomes?

Saravelos Sotirios; Balfoussia Danai; Sarodey Geet; Manukian Barbara; Lavery Stuart; Salim Rehan

Imperial College Healthcare NHS Trust

Objective: The objective of the study was to ascertain whether women with a predicted poor ovarian response based on antral follicle count (AFC) experience poor clinical outcomes.

Methods: This was a retrospective database analysis of prospectively collected data from a tertiary University Fertility Clinic between the years of 2016-2018. AFC in all cases was performed in-house using GE Voluson series ultrasound machines (GE Medical Systems Kretztechnik GmbH & Co, Austria). AFC, ovarian response and clinical outcomes were all entered into a dedicated clinic database. Data was retrieved and analysed using SPSS V25. Mean $\pm$ standard deviation (SD) was used to express continuous variables, while scatter plot graphs with regression fit lines (LOESS) and bivariate Pearson correlations were performed to ascertain their associations. Crosstabulation with Chi-squared tests and binary logistic regression analyses were performed to ascertain associations between AFC and clinical outcomes.

Results: In total 1768 cases were available for analysis. Pearson correlation demonstrated a significant association between AFC, age and number of eggs retrieved ($P < 0.001$). The mean ($\pm$ SD) number of eggs retrieved according to the AFC of women were: 9.5 ± 6.9 (AFC < 5); 12.2 ± 7.2 (AFC 5-10); 17.0 ± 7.5 (AFC 11-20); and 22.6 ± 10.2 (AFC > 20) ($P < 0.001$). For women ≤ 37 years, the pregnancy rates resulting from their first embryo transfer according to AFC were: 45.3% (AFC

<5); 49.2% (AFC 5-10); 50.3% (AFC 11-20); and 58.8% (AFC >20) (Pearson Chi-Square $P=0.224$; Mantel-Haenszel test of trend $P=0.050$). Binary logistic regression found age to be a predictor of pregnancy ($P=0.002$), but not egg number ($P=0.543$) or AFC ($P=0.159$).

Conclusion: Although AFC is associated with the number of eggs retrieved, it is not independently associated with the chance of pregnancy. Women with an AFC <5 appear to have favourable clinical outcomes, both in terms of number of eggs retrieved and pregnancy rate. This has significant implications for patient counselling. Future studies should focus on the relation between AFC and cumulative pregnancy rates, which may prove to be different.

P138 Should patient age be considered within an elective single embryo transfer (SET) policy?

Jacques Celine; Barkaoui Samya; Kotrotsou Mara; Beaumont Lucy; Louzada Julio; Noublanche Caroline; Hickman Cristina

Apricity

Introduction: Clinics, CCGs, and NICE have devised elective (e)SET policies to reduce the risk of multiple pregnancy, the biggest risk associated with IVF, with patient age as a determinant. The objective was to identify the repercussions of age on chance of overall live birth rate per cycle started (LBR) and multiple live birth (mLB) rates following single (SET) and multiple (MET) embryo transfer.

Methods: Population based cohort study using the HFEA register (2010 to 2016). Fresh cycles with own eggs, own sperm were included for female age cohorts 18-34($n=140874$ cycles), 35-37($n=72209$ cycles), 38-39($n=49331$ cycles), 40-42($n=42518$ cycles), 43-44 ($n=11097$ cycles) and 45-50 ($n=3409$ cycles).

Results: SET occurred in a minority(45%) of fresh cycles across all ages. Proportion of MET increased with age (18-34 to 45-50: 46%,54%,65%,71%,70%,60%). Risk of mLB following MET decreased with age, but only marginally (from 1.4 LBR in patients under 35, to 1.1 in patients 45-50). Overall, patients under 35 did not benefit from MET (SET vs MET LBR: 36%, $n=29763$ vs 36%, $n=25652$, NS,Odds Ratio,OR,1.0 $\pm$ 0.02 CI). Older patients only had a marginal increase in LBR, which was constant with increasing age (35-37:3%,OR1.6 $\pm$ 0.03; 38-39:5%,OR1.3 $\pm$ 0.04;40-42:5%, OR1.4 $\pm$ 0.09; 43-44:2%,OR1.4 $\pm$ 0.28; 45-50:2.5%,OR1.8 $\pm$ 0.22).

Conclusion: Age should not be considered within an eSET policy. Literature suggests embryo quality is a better determinant of the number of embryos for transfer. Overall, MET provided no benefit to young patients, and only a marginal benefit in terms of increased live birth rate compared to SET. This marginal increase should be counselled to patients within the context of the increased risks associated with multiple pregnancies.

P139 The effect of equilibration time on pregnancy rate in frozen-thawed embryo transfer cycles

Movahedin Mansoureh¹; Omid Marjan²; Shirzad Negin²; Salehpour Saghar³

¹Tarbiat Modares University; ²Gandhi Hospital, Tehran, Iran; ³Shahid Beheshti Medical Sciences, Tehran, Iran

Background: Embryo re-expansion time in vitrification process is one of the factors that affect the clinical outcomes in the in-vitro fertilization (IVF) programs.

Objective: The main goal was to evaluate the effect of re-expansion time on pregnancy rate in frozen-thawed embryo transfer (FET) cycles.

Materials and Methods: Embryos were vitrified at day three using vitrification method. Dimethyl sulfoxide (7.5%) and ethylene glycol (7.5%) were used for equilibration solution. Seventy-four frozen-thawed embryo transfer cycles were divided into three groups of A) less than 8 minutes, B) 8 to 10 minutes, and C) more than 10 minutes. Maternal age, number of cumulus-oocyte complexes, number of mature oocytes, rates of fertilized oocytes, embryo formation, and embryo quality were compared between different groups. Embryo warming was performed on day three and warmed embryos were cultured till day five and good quality blastocysts were selected for embryo transfer.

Results: There were no significant differences for maternal age, number of retrieved oocytes, mature oocytes between different groups. The rates of fertilized oocytes, embryo formation, embryo quality, number of transferred embryos were the same in different groups. Rate of pregnancy had an increasing trend in group C compared to group A (51.4% and 33.3%, respectively), but the difference was not significant.

Conclusion: The equilibration time may influence on pregnancy rates in FET cycles, however more studies with larger sample size are required. Keywords: Re-expansion time, Vitrification, Pregnancy.

P140 Morphological abnormalities of equine fetuses obtained from mares presenting with early pregnancy loss

Kahler Anne; McGonnell Imelda M.; Smart Harriette; Smith Ken C.; Wathes D. Claire; de Mestre Amanda M

The Royal Veterinary College, UK

Early pregnancy loss (EPL) is of significant importance in equine reproductive medicine but the aetiology is mostly unknown. Fetal abnormalities associated with early abortions in other species are not defined in the equine. We hypothesized that morphological abnormalities present in fetuses from EPL but not clinically normal pregnancies (CNP). Our objective was to compare the gross and histological morphology of EPL and CNP fetuses and the published literature. CNP were obtained following pregnancy termination (PPL70/8577) and EPL submitted by attending veterinary surgeons (URN2012/1169&URN2017--1660-3). Macroscopic morphology and developmental age were determined independently by three blinded examiners, using photographs of fetuses and a standardized protocol. The CRL was measured (CNP=10; EPL=20) in ImageJ. Sagittal sections (6µm) of fetuses were Hematoxylin-Eosin stained (CNP=6; EPL=13) and assessed microscopically. $P<0.05$ (Fishers Exact test) was deemed significant and correlations expressed by Pearson coefficient (GraphPad). Gestational age and CRL were strongly positively correlated in reference1 and CNP ($n=15$, $R=0.93$, $P<0.0001$) but not EPL ($R=0.12$, $P=0.64$), including EPL with alignment between gestational and developmental age ($n=17$). Intrauterine growth retardation (IUGR) was defined as smaller than the 10th-percentile of the CRL of age-matched reference and CNP. Of EPL with measurable CRL and IUGR-reference available ($n=7$), 2 appeared growth retarded. Age and CRL did not correlate following exclusion of IUGR fetuses ($n=15$, $R=0.17$, $P=0.55$) and a mismatch of fetal size and age was interpreted as morphological feature of EPL. Macroscopic abnormalities of the central nervous system (CNS) were identified in EPL and malformations confirmed histologically in one fetus, suffering failed neural tube closure. In the remaining EPL but not CNP, the neural tissue presented with non-specific cellular/dense vs. acellular/cavernous alterations, associated with extracellular oedema and/or hemorrhage. Results provided evidence for IUGR and mismatch of fetal size and age associated with equine EPL. The CNS was commonly disrupted in EPL.

1Jenner F, Närviäinen J, de Ruijter-Villani M, Stout TAE, van Weeren PR, Brama P. Magnetic resonance microscopy atlas of equine embryonic development. Equine Veterinary Journal. 2014;46(2):210-215.

P141 Differential metabolic profiles of lactobacillus species and preterm birth-associated bacteria

Amabebe Emmanuel; Anumba Dilly; Reynolds Steven

University of Sheffield

Introduction: Infection and preterm birth (PTB) associated bacteria in the vaginal microbiome seek to gain an advantage over protective Lactobacillus species, which increases the risk adverse pregnancy outcomes (1). ^1H -Magnetic Resonance Spectroscopy (MRS) has been used to profile the metabolome of cervicovaginal fluid and its relationship to PTB (2, 3). MRS can also be used to track cellular metabolism of multiple ^{13}C -labelled substrates (4). We examined whether different Lactobacillus species and PTB-associated bacteria (Gardnerella vaginalis) differentially metabolise glucose, L- and/or D-lactate using ^{13}C -MRS.

Methods: Lactobacillus species (L. crispatus and L. jensenii), and G. vaginalis were cultured at 37°C under anaerobic conditions (10% CO_2 , 80% N_2 , 10% H_2 ; Lactobacilli: MRS broth, 24h; Gardnerella, BHI broth, 48h). Subsequently, bacteria were subcultured with either $^{13}\text{C}_6$ -glucose, $^{13}\text{C}_3$ -D-lactate or $^{13}\text{C}_3$ -L-lactate (or combined ^{13}C -glucose and ^{13}C -L/D-lactate) for a further 24 and 48h as above. ^{13}C -spectra were acquired using a 9.4T MRS spectrometer. Broth only and bacterial samples with no ^{13}C -substrates were used as controls.

Results: ^{13}C -spectra from *L. crispatus* and *L. jensenii* incubations showed conversion of glucose (natural abundance and ^{13}C -labelled) to lactate and a small amount of acetate. Incubating with $^{13}\text{C}_3$ -lactate only (either L or D enantiomer) showed higher conversion to acetate by *L. jensenii* than *L. crispatus* (also observed for $^{13}\text{C}_u$ -glucose plus $^{13}\text{C}_3$ -L/D-lactate). Succinate was observed in *L. jensenii* ^{13}C -spectra, potentially metabolised from broth. For *G. vaginalis* ^{13}C -glucose incubations, the spectra showed more prominent acetate peaks as well as peaks for succinate, formate and ethanol, but no evidence for lactate metabolism (L or D enantiomer).

Conclusion: Lactobacilli and *G. vaginalis* present in the vagina differentially metabolise ^{13}C -labelled glucose and lactate. The conversion of lactate to acetate and succinate by *L. jensenii* and *G. vaginalis* compared to *L. crispatus*, suggests a possibly important pathomechanism of dysbiosis, altered vaginal pH and infection-associated spontaneous PTB.

1. Amabebe E, Anumba DOC. The Vaginal Microenvironment: The Physiologic Role of Lactobacilli. *Front Med (Lausanne)*. 2018;5:181.
2. Amabebe E, Reynolds S, Stern VL, Parker JL, Stafford GP, Paley MN, et al. Identifying metabolite markers for preterm birth in cervicovaginal fluid by magnetic resonance spectroscopy. *Metabolomics*. 2016;12:67.
3. Stafford GP, Parker JL, Amabebe E, Kistler J, Reynolds S, Stern V, et al. Spontaneous Preterm Birth Is Associated with Differential Expression of Vaginal Metabolites by Lactobacilli-Dominated Microflora. *Frontiers in Physiology*. 2017;8(615).
4. Calvert SJ, Reynolds S, Paley MN, Walters SJ, Pacey AA. Probing human sperm metabolism using ^{13}C -magnetic resonance spectroscopy. *Mol Hum Reprod*. 2019;25(1):30-41.

P143 The prevalence of chromosomal abnormalities in couples experiencing recurrent miscarriages

Mehboob Neha¹; Dixit Abhijit²; Martin Katherine²; Jayaprakasan Kanna³

¹University of Birmingham; ²University Hospitals of Nottingham; ³University of Nottingham

Background: Approximately 1% of couples experience recurrent miscarriage. While 60% of any spontaneous miscarriage is caused by chromosomal abnormalities, most are sporadic and related to age. ESHRE recommends parental karyotype after individual assessment of the risk. This study aims to estimate the prevalence of chromosomal abnormalities in both partners in order to aid in accurate counselling.

Methods: This observational study was undertaken in a tertiary referral centre. Online medical records and databases were used to obtain prospectively collected data of all patients with a history of recurrent miscarriages that underwent karyotype testing between January 2015 to December 2018. The prevalence of chromosomal abnormalities was then calculated amongst the population of eligible patients. Chi-square test was used to compare the prevalence between female and male partners.

Results: A total of 1062 participants (576 women and 486 male partners) had karyotype testing during the study period. There were 11 samples (5 female and 6 male samples) excluded due to various reasons - duplicate records (5), failed karyotype (2) and insufficient data recorded (4). 1.8% (19/1051) of the over-all population had a chromosomal abnormality. The chromosomal abnormalities detected were balanced reciprocal translocations (68.4%; 13/19), paracentric inversions (21%; 4/19), Robertsonian translocation (5.3%; 1/19) and variant mutations (5.3%; 4/19). The prevalence of chromosomal abnormalities was similar in women (1.8%; 10/571) and men (1.9%; 9/480) tested ($P=0.88$).

Conclusion: The prevalence of parental chromosomal abnormalities in those experiencing recurrent miscarriages was found to be 1.8% with balanced translocation abnormalities being the commonest. Both female and male partners had similar rates of abnormalities. This data aids in counselling women or couples who are undergoing karyotyping about the prevalence of chromosomal abnormalities.

P144 ICSI does not improve reproductive outcomes in varied ovarian response cycles in the absence of male factor subfertility: analysis of 327,979 index ART treatment cycles

Supramaniam Prasanna Raj¹; Lim Lee Nai¹; Granne Ingrid²; Ohuma Eric³; McVeigh Enda²; Venkatakrishnan Radha⁴; Becker Christian²; Mittal Monica⁵

¹Oxford University Hospital NHS Foundation Trust; ²Oxford Endometriosis Care Centre, Nuffield Department of Women's and Reproductive Health, University of Oxford; ³Centre for Tropical Medicine and Global Health, Nuffield Department of

Background: ICSI is standard practice for severe male factor subfertility and is associated with improved reproductive outcome in this cohort of patients. The use of ICSI appears to be increasing with the rationale of improved fertilisation rate. There are limited studies evaluating the impact of method of fertilisation based on oocyte yield.

Methods: All cycles recorded on the anonymised Human Fertilisation and Embryology Authority database between 1991-2016 were analysed retrospectively. All first fresh cycles with normal sperm parameters were included. Cycles using donor gametes and cycles utilising preimplantation genetic testing were excluded. A total of 327,979 fresh cycles fulfilled the inclusion and exclusion criteria. Of these, 178,345 (54.4%) were IVF cycles and 149,634 (45.6%) ICSI cycles. Patients were divided into 6 groups based on their ovarian response: Group 1: 1-3 oocytes, Group 2: 4-9 oocytes, Group 3: 10-15 oocytes, Group 4: 16-20 oocytes, Group 5: 21-25 oocytes, Group 6: >25 oocytes. Logistic regression was performed and a confidence interval of 99.5% was used to avoid clustering of cycles as we were unable to link the cycles to the individuals¹. Statistical significance: $p < 0.005$ using Logistic Regression.

Results: The overall live birth (LB) is not improved with ICSI (adjusted OR: 0.99, 99.5% CI 0.96-1.01, $p = 0.115$) compared to conventional IVF, after adjusting for potential confounders. In Group 1, ICSI did not confer any benefit in the LB (aOR: 0.97, 99.5% CI 0.88-1.08, $p = 0.461$) compared to conventional IVF. Similar findings were demonstrated in Group 3 (aOR: 1.00, 99.5% CI 0.96-1.04, $p = 0.833$), Group 4 (aOR: 1.01, 99.5% CI 0.94-1.08, $p = 0.750$), Group 5 (aOR: 0.98, 99.5% CI 0.88-1.09, $p = 0.624$) and Group 6 (aOR: 1.10, 99.5% CI 0.93-1.30, $p = 0.120$). ICSI, however, demonstrated a 4% reduction in the LB in Group 2 (aOR: 0.96, 99.5% CI 0.93-0.99, $p = 0.003$) compared to IVF.

Conclusions: This is the largest retrospective study to date evaluating this study question. ICSI does not confer any benefit in the LB outcome when compared to IVF in all groups of ovarian response except in women where 4-9 oocytes were retrieved with a 4% reduction in LB outcome seen with ICSI.

¹Sunkara Kamal, S., Khalaf, Y., Maheshwari, A., Seed, P., & Coomarasamy, A. (2014). Association between response to ovarian stimulation and miscarriage following IVF: an analysis of 124 351 IVF pregnancies. *Human Reproduction (Oxford, England)*, 29(6), 1218.

P145 Effect of post-thaw processing conditions on human ovarian stroma

Bjarkadottir Briet¹; Boskovic Nina¹; Adeniran Babatomisin¹; Lane Sheila²; Williams Suzannah¹

¹Nuffield Department of Women's and Reproductive Health, University of Oxford, Oxford, UK; ²Department of Paediatric Oncology and Haematology, Children's Oxford University Hospitals NHS Foundation Trust, Oxford, UK

With increasing childhood cancer survival rates there is a greater need to offer appropriate treatments for fertility preservation. For prepubertal girls, ovarian tissue can be cryopreserved for subsequent reimplantation. However, reimplantation is not appropriate for those with haematological malignancies, whereas generating mature oocytes from cultured cryopreserved ovarian tissue would provide fertility preservation. Ovarian tissue cryopreservation and thawing protocols are optimised for follicle health and thus, focusing on the ovarian stroma, that supports follicle development, could improve outcomes of in vitro culture. This study aimed to assess the effects of medium temperature and albumin concentration on stromal cells during processing of cryopreserved-thawed human ovarian tissue.

Cryopreserved cortical strips from post-pubertal women (aged 18-30 years; $n = 3$) were allocated to four groups: pre-cooled (4°C) media with either 3 or 10 mg/mL human serum albumin (HSA) or pre-warmed (37°C) media with either 3 or 10 mg/mL HSA. Thawed strips were processed into small pieces ($< 1 \text{ mm}^3$) and fixed either immediately after thawing, after 2h of processing or after 24h of culture. Masson's trichrome staining was used to measure collagen-enriched stromal area, while TUNEL was used to assess stromal cell death.

Collagen-enriched area was not affected by the conditions tested at any time-point. Whereas stromal cell tissue death was significantly lower after 2 hours of processing in pre-warmed medium with 3 mg/mL HSA ($23.1\% \pm 6.7$) compared to pre-cooled medium with 3 mg/mL HSA ($40.0\% \pm 12.5$, $p < 0.05$) and 10 mg/mL HSA ($37.2\% \pm 11.6$, $p < 0.05$). There was no significant difference in stromal cell death between conditions after thawing or after 24h culture.

Stromal cell death in cryopreserved ovarian tissue can be reduced by altering the processing conditions. Therefore, more focus on improving stroma health in cultured cryopreserved ovarian tissue may lead to higher rates of follicle, and thus egg, development in vitro.

P146 Morphological and molecular assessment of cryopreserved bovine ovarian tissue

Cornally Paula; Longhurst Georga; O'Connor-Moneley James; O'Shea Lynne

University College Dublin

Introduction: Ovarian tissue cryopreservation is gaining considerable interest as a potential option for fertility preservation, particularly in female cancer patients. It is highly relevant to patients with time and hormone sensitive malignancies and is the only viable option for prepubertal girls. Since 2004, over 100 live births and pregnancies have been reported using this technique. Aim of investigation: To determine the effect of bovine ovarian tissue cryopreservation on follicle morphological integrity and key hormonal signalling pathways.

Methods: Bovine ovarian cortical sections were randomly distributed into two cohorts of fresh and cryo-thawed tissue for parallel analytical comparison (n=7). Cryopreserved tissue was supplemented with cryoprotectant and slow-frozen in a Planner Cryo-freezer. Tissue was thawed in media containing decreasing concentrations of ethylene glycol. Fresh and cryo-thawed tissue was cultured for 14 days, with culture media and tissue samples analysed for regulation of key hormonal signalling pathways using ELISA, immunoblotting and immunohistochemistry.

Results: Histological examination showed that cryopreservation of bovine ovarian tissue had a significant effect on follicle viability ($p<0.05$). In addition, cryo-thaw of ovarian tissue caused a significant decrease ($P<0.05$) in progesterone secretion versus the fresh control; however, it had no effect on 17β -estradiol levels. Molecular analysis showed that nuclear progesterone receptor and AVEA protein expression is decreased after ovarian tissue following cryopreservation.

Conclusion: In the present study we determined that cryo-thawing of ovarian tissue has a significant effect on follicle morphology and protein expression. Acknowledgements: This work was supported by UCD Foundation. The authors have no financial disclosures.

P147 Optimising the differentiation of granulosa cells from mouse embryonic stem cells

Watson Lucy¹; Hardy Kate²; Childs Andrew²

¹Royal Veterinary College, UK; ²Imperial College London, UK

Background: Early granulosa cell (GC) development is poorly understood, but requires Wnt signalling and the GC-specific transcription factor FOXL2. Embryonic stem cell (ESC)-derived GCs could provide an in vitro model of GC development, but existing derivation protocols are inefficient. Here, we have explored whether manipulating Wnt signalling and FOXL2 expression in mouse (m)ESCs could enhance GC derivation.

Methods: E14TG2a mESCs were i) treated with $8\mu\text{M}$ CHIR99021 or vehicle (DMSO) without LIF for 3 days(d), then allowed to differentiate for 9d, or ii) transfected with pCMV6-FOXL2-FLAG or empty vector and maintained in 2i+LIF media. Gene and protein expression were assessed by RT-qPCR and immunoblotting.

Results: We assessed the effect of Wnt-signalling activator (GSK3 β inhibitor) CHIR99021 on mESC differentiation towards GCs. At d3 of differentiation, CHIR99021 increased Brachyury (T; mesendoderm marker) expression (18.58 ± 5.4 -fold vs controls, $P=0.01$), and reduced Oct4 (Pou5f1; pluripotency) expression ($31.9\pm 9.4\%$ of controls, $P=0.02$). CHIR99021 increased Osr1 (intermediate mesoderm; 34.6 ± 6.8 -fold vs d0 levels, $P=0.02$) at d8 and Wt1 (gonadal ridge; 2.01 ± 0.2 -fold vs d0, $P=0.03$) at d12. No change was observed in vehicle-treated controls. Upregulation of Wnt4 was observed (gonadal ridge; 1.91 ± 0.2 -fold vs d0) at d12, but did not reach significance.

To explore reprogramming mESCs into GCs, we overexpressed FOXL2. Exogenous FOXL2 levels fell to $30.7 \pm 9.8\%$ and $1.28 \pm 0.66\%$ of 24h levels by 48h and 72h post-transfection, respectively. Expression of Fst, a known FOXL2 transcriptional target in GCs, was upregulated in FOXL2-transfected cells (1.49 ± 0.04 -fold at 72h vs controls). OCT4 expression remained unchanged, demonstrating the effect of FOXL2 to be gene-specific.

Conclusions: CHIR99021 enhances mESC differentiation into mesendoderm, and possibly intermediate mesoderm. As these tissues form the gonadal ridge in vivo, CHIR99021 treatment may improve GC derivation from mESCs. Activation of endogenous GC-expressed genes in mESCs through FOXL2 overexpression suggests reprogramming using GC-specific transcription factors could be an efficient approach to GC derivation.

P148 A proposed national guideline for the management of ovarian torsion

Farahani Linda; Wali Sarah; Mittal Monica; Rai Raj; Salim Rehan; Webber Lisa

Imperial College Healthcare NHS Trust

Background: Ovarian torsion (OT) is responsible for 2.7-7.4% of gynaecological emergencies and most commonly affects women of reproductive age. Available evidence recommends conservative surgical management to preserve the ovary and fertility, but without a national UK guideline are clinicians managing cases in the most appropriate way?

Methods: Literature review conducted using the terms "ovarian", "adnexal", "torsion". An audit of all cases of OT between 2015-2019 at our local unit.

Results: 30 confirmed cases of OT, managed with oophorectomy in 11 cases (37%), cystectomy in 16 cases (53%), and detorsion only in 3 cases (10%). There were no reported complications following conservative management. The findings of the literature review show that blue-black necrotic-appearing ovaries recover^{1,2,3,4}(visually and functionally) when detorsion alone is performed. Importantly, in cases of detorsion there were no documented reports of thromboembolic events or intra-abdominal sepsis^{5,6} Reports of oophoropexy in the literature are rare, but there is evidence that ovarian ligament length is correlated with risk of torsion, and oophoropexy in select cases may help reduce recurrence⁷

Conclusion: The audit highlights that despite available evidence, radical surgery is regularly performed in women of reproductive age. Guideline recommendations 1. Laparoscopy is the surgical approach of choice 2. Conservative surgical management should be performed in premenopausal women (either detorsion alone, or detorsion and cystectomy) 3. Clinicians should consider detorsion only if there is no previous history of known non-functional ovarian cysts (e.g. mature cystic teratoma) 4. Clinicians should consider an interval ovarian cystectomy in patients that require it in order to avoid operating on an ovary that is oedematous and friable. The exception to this would be in cases where the ovary is not oedematous intra-operatively 5. An oophorectomy should be performed only in a postmenopausal patient because of the potential risk of malignancy, or in cases where it is unavoidable

1. Geimanaite L, Trainavicius K. Pediatric ovarian torsion: Follow- up after preservation of ovarian tissue. J Pediatr Surg. 2019 Jul;54(7):1453-1456. doi: 10.1016/j.jpedsurg.2019.02.004. Epub 2019 Feb 22. PubMed PMID: 30851955. 2. Oskayli MC, Gulcin N, Ozatman E, Gercel G, Mutus M, Aksu B, Durakbasa CU. Assessment of ovarian reserve using serum anti-Müllerian hormone after ovarian torsion surgery. Pediatr Int. 2019 May;61(5):504-507. doi: 10.1111/ped.13818. Epub 2019 May 17. PubMed PMID: 30825401. 3. Resapu P, Rao Gundabattula S, Bharathi Bayyarrapu V, Pochiraju M, Surampudi K, Dasari S. Adnexal torsion in symptomatic women: a single-centre retrospective study of diagnosis and management. J Obstet Gynaecol. 2019 Apr;39(3):349-354. doi: 10.1080/01443615.2018.1494702. Epub 2018 Nov 8. PubMed PMID: 30406700. 4. Walker SK, Lal DR, Boyd KP, Sato TT. Management of pediatric ovarian torsion: evidence of follicular development after ovarian preservation. Surgery. 2018 Mar;163(3):547-552. doi: 10.1016/j.surg.2017.11.019. Epub 2018 Jan 9. PubMed PMID: 29329768. 5. Wang Z, Zhang D, Zhang H, Guo X, Zheng J, Xie H. Characteristics of the patients with adnexal torsion and outcomes of different surgical procedures: A retrospective study. Medicine (Baltimore). 2019 Feb;98(5):e14321. doi: 10.1097/MD.00000000000014321. PubMed PMID: 30702612; PubMed Central PMCID: PMC6380708. 6. Adeyemi-Fowode O, Lin EG, Syed F, Sangi-Haghpeykar H, Zhu H, Dietrich JE. Adnexal Torsion in Children and Adolescents: A Retrospective Review of 245 Cases at a Single Institution. J Pediatr Adolesc Gynecol. 2019 Feb;32(1):64-69. doi: 10.1016/j.jpog.2018.07.003. Epub 2018 Nov 12. PubMed PMID: 30012428. 7. Gonçalves IS, Sampaio J, Félix J, Silva AC, Fornelos G, Silva PT. Oophoropexy to the Round Ligament after Recurrent Adnexal Torsion. Rev Bras Ginecol Obstet. 2018 Nov;40(11):726-730. doi: 10.1055/s-0038-1673426. Epub 2018 Oct 11. PubMed PMID: 30308681.

P149 Ovarian histomorphometry and follicular development in pre-pubertal albino rats with dietary vitamin d supplementation

Mbegbu Edmund¹; Obidike Ikechukwu¹; Childs Andrew²; Fouladi-Nashta Ali³

¹University of Nigeria, Nsukka; ²Imperial College, London, UK; ³Royal Veterinary College

Folliculogenesis is a key event in female reproduction, involving successive transformation of primordial follicles containing immature primary oocyte in order to produce a viable gamete. The role of vitamin D in ovarian follicles development and fertility has been suggested based on wide range of scientific and clinical observations. Eighteen pre-pubertal female albino rats, 3-4 weeks of age, weighing 70.25 ± 9.16 grams, assigned to three groups Group A (control): received 5 ml/kg of distilled water, Group B received 0.025mg/kg of vitD3 dissolved in distilled water, Group C received 0.125mg/kg of vitD3 dissolved in distilled water. The rats were housed in metal cages at room temperature (27-32°C) in a room devoid of direct or reflective UV rays from the sun. All treatments were orally administered twice weekly for 28 days when blood and ovaries were harvested under anaesthesia (n=6). Plasma concentrations of vitD3 was determined using spectrophotometric method. The ovaries were fixed in Bouin's fluid, serially sectioned at 5 microns and every 10th H&E stained-section selected for histomorphometry after imaging. Number of follicles at each developmental stage were first counted with cell counter tool on ImageJ software (version 1.48; NIH), and subsequently estimated using the formula: $N_t = N_f \times S_t \times t_s / (S_o \times d_o)$. Group A (control) had lower ($P < 0.05$) vitD3 level, fewer ($P < 0.05$) growing follicles (primary, secondary and antral follicles) and more ($P < 0.05$) non-growing follicles (primordial and atretic follicles) compared with the vitD3-supplemented Groups. The ovarian surface area however decreased with increasing vitD3 supplementation. Findings from this research indicate that vitD supplementation could enhance optimal ovarian follicle recruitment and development in female rats. Key words: Fertility, Histomorphometry, Ovary, Rats, Vitamin D

P150 Determining frozen ovarian cortical tissue composition after enzymatic dissociation

Adeniran Babatomisin¹; Papadouloupou Eleni¹; Lane Sheila²; Williams Suzannah¹

¹University of Oxford; ²Oxford Reproductive Tissue Cryopreservation Programme & NHS Foundation Trust, Oxford

Cancer survival rates for children are increasing and therefore we need to improve methods for fertility preservation as well as develop new techniques. For patients that require gonadotoxic treatments, ovarian cortical tissue can be cryopreserved to preserve fertility. However, since cellular composition within the ovary is highly heterogeneous, it is important to understand more about the tissues that have been cryopreserved. By digesting these tissues into a single cell population, we can readily analyse the different populations contained within. Therefore, the first step is developing a method of tissue dissociation that is optimal for maximizing cell numbers as well as cell viability. This study aimed to investigate the ability of different enzymatic conditions to dissociate human ovarian tissue into single cells. Frozen ovarian cortical strips from post-pubertal patients (18--29 years; n=3), were weighed and then dissected into $< 1 \text{ mm}^3$ pieces. Tissues were subjected to three different dissociation methods: at 37°C using 3mg/ml Collagenase I and 0.078mg/ml DNAase I either in L-15 or PBS stationary, or for 1 hour or in L-15 on a shaker for 40 minutes. The number of live and dead cells were determined using trypan blue and the number of cells using a hemocytometer. The total number of cells obtained in the L-15-based enzymatic media either with or without the shaker did not differ ($1.3 \times 10^8 \pm 7.2 \times 10^6$ versus $1.3 \times 10^8 \pm 1.6 \times 10^7$, respectively) whereas, tissue incubated in PBS resulted in a 61% decrease in cell numbers ($4.8 \times 10^7 \pm 1.2 \times 10^7$; n=3). However, of the cells obtained following digestion in L-15 media the largest proportion of dead cells were observed in the L-15 shaker condition ($p < 0.05$; n=3). We can conclude that tissue dissociation in stationary L-15 enzymatic media was the most effective in maximizing the number of live cells obtained. Using this method, we can advance towards investigating the various cell populations.

[1] Donnez J., Dolmans M. Fertility preservation in women. The New England journal of medicine 2017; 377(17): 1657-1665 [2] Gougeon A. Human ovarian follicular development: from activation of resting follicles to preovulatory maturation. Ann Endocrinol (Paris) 2010; 71(3): 132-43.

P151 Mid-cycle scans in controlled ovarian stimulation, are they needed? A re-audit from a large IVF centre

Nancarrow Lewis; Twist Benjamin; Drakeley Andrew

Hewitt Fertility Centre

Purpose: Scanning takes up a large proportion of an IVF centre's workload. This audit's aim was to see if there day 6 follicular tracking scans are needed and whether they influence IVF outcomes.

Methods: 100 mid-stimulation scans were reviewed over a 53-day period. Changes in treatment, oocyte numbers, cases of ovarian hyperstimulation syndrome (OHSS) and clinical pregnancy rates were noted. The following year a re-audit was done over the same time period.

Results: 71/100 patients were scanned due to their risk of developing OHSS and whether a stimulation reduction at mid-cycle scan would reduce this. 0/100 patients had a dose reduction, 1 had an earlier egg collection on day 10 and 15 patients had a stimulation dose increase. 17 patients required GnRH-agonist triggers due to risk of OHSS. There was 1 case of mild OHSS and 1 moderate OHSS. The initial audit determined that day 6 scans were not required for those deemed at risk of OHSS and who were on the lowest dose of stimulation. However mid-cycle scans were appropriate for those where dose adjustment is possible, particularly perceived poorer responders. Over the same time period, 1 year later, only 55 patients had a mid-cycle scan. Only 4 had an increase in stimulation dose with one patient having a dose reduction. There were no OHSS cases in re-audit.

Conclusion: Use of mid-cycle scans rarely leads to management changes in controlled ovarian stimulation cycles. They should be reserved for those who have scope for change in their stimulation dose or whose antral follicle count at time of baseline scan contradicts their prescribed stimulation dose. Reduction in scans can relieve pressures on a busy unit and also reduced number of appointments and costs that can burden patients during their treatment cycles. However this can lead to patients feeling less cared for.

P152 Do environmental contaminants alter oviduct epithelial nutrient transport?

Papachristofi Vasiliki ¹; Rotchell Jeanette ²; Sturme Roger G ¹

¹Hull York Medical School, University of Hull; ²University of Hull, Department of Biological and Marine Sciences

Purpose: Bisphenols are a class of chemicals used for the production of plastics and resins. Bisphenol A (BPA) and bisphenol S (BPS) are present in the environment resulting in exposure in wildlife and humans. These chemicals have been detected in human fluids, serum(1), urine(2), breast milk(3) and in cord serum(4). Relevantly, in vitro studies involving preimplantation embryo development has been shown to be affected by exposure to BPA(5). However, the extent to which the periconceptual environment is sensitive to bisphenols is less well understood. The aim of this study was to use an in vitro preparation of oviduct epithelia to examine the extent to which the nutrient secretion by these cells is sensitive to such environmental contaminants.

Methods: Epithelial cells were isolated from bovine oviducts and cultured in the presence of BPA and BPS for 7 days. One "high" and one "environmentally-relevant" dose of BPA (10uM & 10nM) and BPS (1uM & 1nM) were investigated. Samples of culture media were collected at 24-hour intervals and the concentrations of glucose, pyruvate, lactate were determined using microfluorometric methods.

Results: After 144h exposure, glucose depletion by oviduct epithelial cells was not influenced by the presence of BPA or BPS. However, a high dose of BPA caused a significant decrease in pyruvate concentration (0.1 Vs 0.24mM p=0.0007) and a significant increase in lactate concentration (3.54Vs 1.84mM; p=0.0011). Pyruvate and lactate production were not influenced by the presence of BPS at any dose.

Conclusions: Our findings have revealed that the ratio of pyruvate: lactate released by oviduct epithelia is sensitive to high concentrations of BPA, suggesting that the composition of the periconceptual environment may be influenced by bisphenols. However, environmentally-relevant concentrations of BPA and BPS do not appear to alter oviduct epithelial secretion of key metabolites, pointing to a 'buffering'-effect of the oviduct.

References 1. Wan Y, Xia W, Yang S, Pan X, He Z, Kannan K. Spatial distribution of bisphenol S in surface water and human serum from Yangtze River watershed, China: Implications for exposure through drinking water. *Chemosphere*. 2018 May 1;199:595-602. 2. Liao C, Liu F, Alomirah H, Vu S, Loi D, Mustafa I, et al. Bisphenol S in Urine from the United States and Seven Asian Countries: Occurrence and Human Exposures. *Environ Sci Technol*. 2012;46:6860-6. Available from: <https://pubs.acs.org/doi/10.1021/es301334j>. 3. Dualde P, Pardo O, F. Fernández S, Pastor A, Yusà V. Determination of four parabens and bisphenols A, F and S in human breast milk using QuEChERS and liquid chromatography coupled to mass spectrometry. *J Chromatogr B*. 2019 May 1;1114-1115:154-66. Available from: <https://www.sciencedirect.com/science/article/pii/S1570023218318452?via%3Dihub>. 4. Liu J, Li J, Wu Y, Zhao Y, Luo F, Li S, et al. Bisphenol A Metabolites and Bisphenol S in Paired Maternal and Cord Serum. *Environ Sci Technol*. 2017 Feb 21;51(4):2456-63. Available from: <http://pubs.acs.org/doi/10.1021/acs.est.6b05718>. 5. Choi BI, Harvey AJ, Green MP. Bisphenol A affects early bovine embryo development and metabolism that is negated by an oestrogen receptor inhibitor. *Sci Rep*. 2016 Sep 7;6(1):29318. Available from: <http://www.nature.com/articles/srep29318>

P153 Diagnostic accuracy of oxidation-reduction potential (ORP) in predicting the outcome of intrauterine insemination

Wooler Josephine; Meadows Jessica; Sharpe Amy; Wyner Michaela; Turley Rory; Blower Jane; Potdar Neelam

Leicester Fertility Centre

Background: Conventional semen analysis does not assess sperm quality at the molecular level. Factors such as Reactive Oxygen Species (ROS) may cause sperm damage through 'oxidative stress', and have been associated with reduced sperm quality and increased sperm DNA fragmentation (SDF)⁽¹⁾. Higher amounts of ROS have also been found in couples who did not achieve pregnancy after intrauterine insemination (IUI) compared to those that did⁽²⁾. Oxidative stress can be measured through oxidation-reduction potential (ORP) using the MiOXSYS™ system (Aytu BioScience Inc., USA), and sperm DNA fragmentation through chromatin dispersion using the Halosperm G2 test (Halotech®, Madrid).

Aims: To examine the relationship between ORP and clinical pregnancy rate following IUI treatment. To correlate ORP with SDF rates, and ORP and sperm parameters.

Methods: In this prospective study, residual semen from IUI treatment was tested for ORP and a small cohort additionally for SDF. Clinical outcomes were categorised as negative, biochemical pregnancy or clinical pregnancy (confirmed through ultrasound scan).

Results: A sample number of n=50 was tested for ORP, and out of these 8 were additionally tested for SDF. There was no significant correlation between ORP and SDF results ($p=0.5821$). However, ORP was significantly negatively correlated with sperm concentration ($p<0.0001$), progressive motility ($p<0.01$) and normal morphology ($p<0.01$). A cut-off value of $0.5\text{mV}/10^6\text{sperm/mL}$ was reasonable in predicting couples who were unlikely to achieve clinical pregnancy (sensitivity was 55.8% and specificity was 68.3%).

Conclusion: ORP has a complementary role in the assessment of sperm quality. The measurement of oxidation-reduction potential is a useful tool in identifying those couples who are less likely to conceive through IUI treatment. The lack of agreement between ORP and sperm DNA fragmentation may be due in part to the low sample numbers, as well as the subjective nature of assessing sperm through dispersion of chromatin.

1. Agarwal A, Bui AD. Oxidation-reduction potential as a new marker for oxidative stress: Correlation to male infertility. *Investigative and Clinical Urology*. 2017;58(6):385-99.

2. D'Agata R, Vicari E, Moncada ML, Sidoti G, Calogero AE, Fornito MC, et al. Generation of reactive oxygen species in subgroups of infertile men. *Int J Androl*. 1990;13(5):344-51.

P154 A comparative analysis of birth weights following a fresh or frozen blastocyst single embryo transfer

Syed Najma; Naik Rahul; Fryer Hayley; Richardson Lucy; Drinkwater Abbie

Herts and Essex Fertility Centre

Background: Numerous studies have compared the neonatal birth weights (BW) following fresh and frozen embryo transfers (FET) (1, 2 & 3). Some studies have reported increased BW with FET (4) whilst others reported no significant difference (5). The variability in outcomes suggests that further research is needed. Consequently, this study aimed to determine whether there were any differences in neonatal BW of infants conceived following fresh or FET.

Method: A retrospective cohort study compared patients (<38 years) who underwent single embryo transfer (SET) following fresh or frozen cycles. Between September 2016 and June 2018, 2462 patients undergoing fresh transfer and 190 patients undergoing FET were suitable for inclusion. All gamete recipient cycles were included. Neonatal BW, live birth (LB) rate and type of birth (caesarean or vaginal delivery) were compared using Students t-test and Pearson's Chi Squared. Significance was accepted at $P = <0.05$.

Results: The average BW following fresh transfer was 3462g compared to 3052g for FET ($P = 0.0115$). This difference is significant. Interestingly, a higher caesarean delivery rate was observed in the frozen ET group than in the fresh ET

group (62.1% vs. 51.33%; $P=0.034$). No significant difference was observed in LB rate between fresh and frozen embryo transfers ($P=0.60$).

Conclusion: The BW are lower in cycles with fresh blastocyst transfer after controlled ovarian stimulation than in transfers of frozen-thawed embryos in the absence of ovarian stimulation. This finding confirms similar results reported in many retrospective studies. However, no statistical significance in the LB rate between fresh and frozen cycles was observed. The above findings provide reassurance to patients and clinicians regarding BW outcomes following assisted reproduction techniques.

References: (1) Eaton J.L., E.S. Lieberman, C. Stearns, M. Chinchilla, C. Racowsky. Embryo culture media and neonatal birthweight following IVF. Human Reproduction. 2012. 27 (2). 357-379. (2) Kalra S.K, Ratcliffe S.J, Coutifaris C, Molinaro T, and Barnhart KT. Ovarian Stimulation and Low Birth Weight in Infants Conceived Through In Vitro Fertilization. Obstet Gynecol. 2011. 118(4): 863-871. (3) Lukea B, Brown MB, Wantman E, Sternd JE. Siblings conceived with assisted reproductive technology: birthweight and gestation differences in fresh vs frozen cycles. Fertility and sterility. 2015. (104)3: e4. (4) Litzky JF, Boulet S, Esfandiari N, Zhang Y, Kissin DM, Theiler RN, Marsite CJ. Frozen embryo transfer associated with increased birthweight but decreased rate of low birthweight in US full-term singleton infants conceived 2007-2014. 2017. 108(3): e37 (5) O'Neill KE, Lanzendorf SE, Odem RR, Cooper AR. Fresh versus frozen sib-pair analysis of birthweight in singleton livebirths as a result of IVF. 2010. 94(4): S19

P155 Does ovarian response differ with age in women with diminished ovarian reserve?

Antwi-Ahima Gloria; Choudhary Meenakshi

Newcastle Upon Tyne Hospitals NHS Trust

Background: Diminished ovarian reserve (DOR) is the decline in oocyte pool and is associated with poor stimulation response, high cancellation rate and low pregnancy rate. It is a physiological process with advancing maternal age however for some women, this process is accelerated. Aim: To determine whether the ovarian response to stimulation in younger women with DOR is better than that of older women with DOR by comparing the number of oocytes, maturation rate and fertilization rate in women over 38 years old with AMH of $<5.4\text{pmol/L}$ to that of women under 35 years with AMH $<5.4\text{pmol/L}$.

Methodology: Retrospective study looking at women going through their first cycle of IVF/ICSI with AMH $<5.4\text{pmol/L}$ and under 35 years or over 38 years reaching the oocyte collection stage from January 2016 to May 2019 at a tertiary hospital in the United Kingdom. There were 32 patients in the young group (group A) and 28 in the older group (group B).

Results: The average oocyte number was 4.4 ± 3.22 in group A and 4.1 ± 2.78 in group B. The average percentage oocyte maturation was 92.5% for group A vs 85.7% for group B. The average percentage of normal fertilization was 70.4% for group A versus 71.5% for group B. 26 women out of 32 had embryo transfer in group A while 24 out of 28 had embryo transfer in group B. 7 women had a clinical pregnancy in group A versus 4 in group B giving a pregnancy rate of 26.9% and 16.6% respectively.

Conclusion: Younger (under 35) and older (over 38) women with AMH $<5.4\text{pmol/L}$ who reach oocyte collection stage of treatment have comparable outcomes of ovarian stimulation however pregnancy rate per embryo transfer is likely to be better in the younger group.

P156 The atypical profiles of HDP biomarkers in aged pregnancy with hypertensive disorders of pregnancy: analysis of maternal senescence

Furuya Kiichiro¹; Kumasawa Keiichi²; Nakamura Hitomi³; Kimura Tadashi³

¹Nottingham University; ²University of Tokyo; ³Department of Obstetrics and Gynecology, Osaka University Graduate School of Medicine

Background: Advanced maternal age (AMA) has increased due to late marriage and advances in reproductive technology. AMA complicates adverse pregnancy outcomes including hypertensive disorders of pregnancy (HDP), a life-threatening complication for foetus and mother[1, 2]. This complication has defined as placental failure and consequent anti-/angiogenic imbalance, so called "two stage theory"[3]. However, pathological mechanism has been

unclear. We have established the AMA mouse model using aged pregnant mice and demonstrated its phenotypes resembled human AMA[4]. Objective: We investigated serum profiles of HDP in AMA individuals and analyzed the evidence of placental dysfunction due to placental senescence in AMA.

Methods: We use "AMA model mice", established as over six months old pregnant Jcl:ICR mice for study. This model animals has been manifested the same complications phenotypes of human AMA including declining fertility, FGR, and higher rate of IUFD, and HDP. Control mice were defined as 8-13 weeks old pregnant mice. We measured their biomarkers of preeclampsia. We also analyzed whether placental and tissue senescence in AMA model mice.

Results: The significant findings of our investigation are as follows: 1)while HDP was complicated, AMA mice and human HDP patients significantly manifested a low serum soluble fms-like tyrosine kinase-1 (sFlt-1) level in late gestation, 2) the other biomarkers of HDP including endothelin- and VEGF levels in AMA also represented atypical profiles of HDP, and 3) placental senescence and oxidative stress in aged individuals significantly increased in aged trophoblast cells

Conclusion: In conclusion, the aged pregnant model mice which has been represented the resembled phenotypes of human AMA including declining fertility, FGR, IUFD, HDP, manifested the atypical profile of HDP biomarkers. Our findings indicated that these complications can be occurred by not only placental dysfunction but also tissue and vessels senescence.

References: 1. Yogev Y, Melamed N, Bardin R, Tenenbaum-Gavish K, Ben-Shitrit G, Ben-Haroush A. Pregnancy outcome at extremely advanced maternal age. *Am J Obstet Gynecol* 2010;203:558.e551-557. 2. Favilli A, Pericoli S, Acanfora MM, Bini V, Di Renzo GC, Gerli S. Pregnancy outcome in women aged 40 years or more. *J Matern Fetal Neonatal Med.* 2012;25:1260-1263. 3. Maynard SE, Min JY, Merchan J, et al. Excess placental soluble fms-like tyrosine kinase 1 (sFlt1) may contribute to endothelial dysfunction, hypertension, and proteinuria in preeclampsia. *J Clin Invest.* 2003;111:649-658. 4. Furuya K, Kumasawa K, Nakamura H, Nishimori K, Kimura T. Novel biomarker profiles in experimental aged maternal mice with hypertensive disorders of pregnancy. *Hypertens Res.* 2019;42:29-39.

P157 The effect of ovine whole ovary cryopreservation on the oocyte and its developmental potential

Dadson Adwoa; Wang Dian; Hernandez-Medrano Juan; Maalouf Walid

University of Nottingham

Background: Whole ovary cryopreservation and transplantation restores ovarian function and natural fertility resulting in multiple live births in sheep, thanks to the preservation and survival of primordial and preantral follicles [1]. However, the effect of cryopreservation on oocytes from antral follicles and their viability have not been reported. This study investigates the viability and capacity of antral follicle oocytes to mature following whole ovarian cryopreservation.

Method: A total of 420 oocytes (control, CT, n=217 and WOCP, n=203) were aspirated from abattoir-sourced sheep ovaries. After collection, oocytes were classified according to COC morphology into normal or abnormal [2]. Oocytes were matured in vitro and nuclear maturation assessed (DAPI) after 24h. The viability (PI staining) and apoptosis (TUNEL apoptotic index) was also assessed following cryopreservation.

Results: Percentage of COCs classified as normal was similar ($P>0.05$) between WOCP (54.5±12.7%) and CT (54.3±8.0%). Moreover, PI positive oocyte percentages were comparable ($P>0.05$) between WOCP and CT: 32.6% (n=23/70) and 19.8% (n=15/76), respectively. However, nuclear maturation was lower ($p<0.05$) in WOCP (8%, n=1/64) compared to CT oocytes (67%, n=53/65), whereas TUNEL apoptotic index was higher in WOCP oocytes than controls (75.4%, n=52/69 vs. 26.6%, n=17/76).

Conclusions: Oocytes isolated from WOCP antral follicles fail to mature in vitro due to the disorganisation of actin filaments leading to the loss of cumulus cells and high level of induced apoptosis. Keywords: Whole ovary, Cryopreservation, Slow freezing

1. Campbell, B.K., Hernandez-Medrano, J., Onions, V., Pincott-Allen, C., Aljaser, F., Fisher, J., McNeilly, A.S., Webb, R., Picton, H.M., 2014. Restoration of ovarian function and natural fertility following the cryopreservation and autotransplantation of whole adult sheep ovaries. *Human reproduction* 29, 1749-1763. 2. Talukder, M., Iqbal, A., Khandoker, M.A.M., Zahangir Alam, M., 2011. Collection grading and evaluation of cumulus-oocyte-complexes for in vitro maturation in sheep. *Bangladesh Veterinarian* 28. Thibier, M., 2006. Biosecurity and the various types of embryos transferred. *Reproduction in domestic animals = Zuchthygiene* 41, 260-267.

P158 Should clomifene citrate be available in primary care: an in-depth interview study

Garthwaite Heather¹; Stewart Jane²; Wilkes Scott¹

¹University of Sunderland; ²Newcastle Fertility Centre at Life

Background: Use of clomifene citrate for managing ovulatory disorders in primary care has gradually declined over the last two decades; currently, approximately 250 prescriptions are issued by GPs in England each month [1]. This decline has partly been since NICE CG156 has advised the use of transvaginal ultrasound (TVUS) during the first cycle of treatment, to assess for multiple pregnancy risk and allow abstinence from unprotected intercourse where there is multifollicular development [2]. The aim of this study was to explore the preferences of patients, fertility specialists and GPs in terms of where clomifene is prescribed, and how it is monitored.

Methods: We conducted in-depth interviews with three cohorts: patients (n=10), fertility specialists (n=7) and GPs (n=9). Recruitment was an iterative process, guided by ongoing thematic analysis.

Results: While all three cohorts acknowledged that GP treatment might be quicker and easier, there was an overall preference for an expert input and support from a fertility clinic. There was also emphasis on optimising communication and timely referral between primary and specialist care. None of the GP participants had recent experience of prescribing clomifene. Several said they would consider prescribing if robust guidelines and education were in place, however the cohort recognised their limited exposure to fertility issues, and expressed concerns about increasing workload and restricted time in consultations. Patients valued TVUS scanning for reassurance that the treatment was working, and fertility specialists echoed this. Most patients (n=9) said that they would be willing to risk a multiple pregnancy, and both cohorts acknowledged the potential difficulty for patients in abstaining from intercourse following multifollicular development.

Conclusions: The value of a specialist input was a common theme that resonated throughout the three cohorts. Participants valued TVUS monitoring but questioned its specific application in managing multiple pregnancy risk; further research is needed.

[1] Total prescribing for clomifene citrate across all CCGs in NHS England. Available at: <https://openprescribing.net/analyse/#org=CCG&numIds=0605010G0&denom=nothing&selectedTab=summary> (accessed 16th September 2019) [2] National Institute for Health and Care Excellence (2015) Fertility problems: assessment and treatment. NICE guideline (CG156).

P159 CGG-repeat RNA aggregates induce granulosa cell death in a model of Fragile X-associated premature ovarian insufficiency

Rosario Roseanne¹; Roy Choudhury Nila¹; Stewart Hazel¹; Michlewski Gracjan¹; Charlet-Berguerand Nicolas²; Anderson Richard A¹

¹University of Edinburgh; ²Institut de Génétique et de Biologie Moléculaire et Cellulaire

Introduction: Fragile X-associated premature ovarian insufficiency (FXPOI) is caused by expansion of a CGG repeat sequence located in the 5'UTR of the FMR1 gene. Approximately 30% of women who carry the premutation allele (55-200 CGG repeats) develop FXPOI¹. Two mechanisms have been proposed to explain the pathology of the premutation allele: RNA gain-of-function, where CGG-repeat RNA sequesters specific proteins, and translation of CGG repeats into a polyglycine-containing protein, FMRpolyG. Here we investigate whether these mechanisms underlie FXPOI.

Methods: Plasmids expressing premutation length CGG repeat RNAs and FMRpolyG were transfected into human granulosa cell lines (HGrC1 and COV434) and a germ-cell cell line (NT2). Combined RNA in situ hybridisation and immunocytochemistry were used to colocalise CGG RNA and FMRpolyG protein aggregates. FACS was used to assess cell viability. RNA pull-down SILAC mass spectrometry (RP-SMS) was carried out to identify proteins sequestered by CGG RNA aggregates.

Results and Discussion: Following plasmid transfection, FMRpolyG protein aggregates were observed in granulosa cell and germ cell lines, however CGG RNA aggregates were seen in granulosa cell nuclei only. These CGG RNA aggregates

enlarged over 72h causing death in ~40% of cells. Combined RNA in situ hybridisation and immunocytochemistry showed that ~70% of HGrC1 cells had large CGG RNA aggregates without accompanying FMRpolyG protein; this inefficient translation is not observed in a related Fragile X-disorder², thus strongly indicates an RNA gain-of-function aetiology in FXPOI. RP-SMS identified RNA binding proteins sequestered by CGG RNA aggregates in HGrC1 cells, including cell-cycle and apoptosis regulators such as FUS, TAF15 and PA2G4.

Our data provide evidence that intranuclear RNA aggregates formed by the FMR1 premutation transcript sequester and deregulate essential proteins in granulosa cells and are the likely causative agents of the premature follicle depletion in FXPOI.

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1. Welt, C. K., Smith, P. C. & Taylor, A. E. Evidence of early ovarian aging in fragile X premutation carriers. *J. Clin. Endocrinol. Metab.* (2004) 89, 4569-4574
2. Sellier, C. et al. Sam68 sequestration and partial loss of function are associated with splicing alterations in FXTAS patients. *EMBO J.* (2010) 29, 1248-1261

P160 Reproductive outcomes and predictors for success following hysteroscopic tubal cannulation for proximal tubal disease

Dobson Sam¹; Conroy Beth; Skull Jonathan¹; Metwally Mostafa¹; Ola Bola¹

¹Jessop Wing, Sheffield Teaching Hospital

Objective: To investigate the effectiveness of hysteroscopic tubal cannulation (HTC) for the treatment of patients with proximal tubal occlusion (PTO).

Design: A retrospective case series

Setting: A large tertiary referral fertility clinic

Patient(s): All patients who underwent HTC for either unilateral (n= 53) or bilateral PTO (n= 33) between 2009 and 2018.

Intervention: All patients underwent laparoscopic chromopertubation followed by HTC with Cook Novy Cornual Cannulation Catheter (Cook UK©) on the obstructed tube(s). Fertility outcomes were collected for the 12 months following HTC.

Main outcome measures: Live birth rate (LBR). Results: Successful HTC was achieved in 63/86 (73.3%) of patients. Overall CPR and LBR following successful HTC was 26.9% and 22.2% respectively. Patients aged 20-35 years achieved a significantly higher CPR (38.5% vs 8.33%; Odds ratio (OR) 6.875; 95% confidence interval (CI) 1.41-33.54) and LBR (30.8% vs 8.3% OR 5.5; 95% CI 1.118-27.1) compared to women 36-45 years old. There were three miscarriages (17.6%) and one ectopic pregnancy identified (5.9%). Both FSH (Standard deviation (SD)) and Age (SD) were significantly lower in patients who achieved a pregnancy (5.6 (1.64) vs 7.3 (2.8), p = 0.021) and (30.8 (4.76) vs 35.2 (5.42), p = 0.004) respectively.

Conclusion: HTC for PTO is an effective alternative to IVF and should be considered as first line treatment, especially in those who are under 36 years of age, with unilateral PTO and normal day 2 FSH levels.

P161 A computational exploration of extracellular vesicles behaviour in the maternal tract

Morales-Garduno Carlos¹; Walker Dawn²; Fazeli Alireza³

¹Department of Computer Science, The University of Sheffield, UK; ²Department of Computer Science, The University of Sheffield, UK and Insigneo Institute for in silico Medicine, Sheffield UK; ³Department of Pathophysiology, University of Tartu, Estonia and Department of Oncology & Metabolism, The University of Sheffield

Background: Although research in reproduction has been active for many years, there are still underlying processes in need of further understanding. One such process is the potential communication occurring between the female host and the male reproductive cells. In mammals, recent evidence has shown extracellular vesicles (EV) are released by oviduct cells and attach to the male gametes (1,2). Yet, many unknowns prevail about the scope of EV involvement in the fertilization process.

To complement the biological research endeavour, some computational tools are available. Among these, Agent-Based Modelling is well suited for a specific variety of complex systems. This technique aims to uncover the traits underpinning the emergent high-level and system-wide behaviour of a system by focusing on the well-known low-level characteristics of its participants and their mutual interactions and with their environment.

Methods: Using histological images, published data characterizing EV displacement, and experimental data characterizing EV production rates in the oviduct, we developed an agent-based model of oviductal EV to explore their presence at key regions of the oviduct.

Results: Our preliminary results show EV of all sizes tend to have higher concentrations near the epithelial tissue which reduces gradually as the distance increase from the tissue. These higher EVs concentrations may be related to spermatozoa favouring displacement near the epithelial tissue. However, at further distances from the epithelium, the concentration profiles show higher numbers of smaller sized EVs than regions closer to the tissue.

Conclusion and Future Work: We developed a computational model of oviduct extracellular vesicles grounded in biology. This model is being used to investigate EV distribution at key regions of the oviduct. Our future work will extend this model into a multi-scale model of EV-spermatozoa interaction to support researching the role of EV in the grand scheme of the fertilization process.

1. Holt W V., Fazeli A. Sperm selection in the female mammalian reproductive tract. Focus on the oviduct: Hypotheses, mechanisms, and new opportunities. *Theriogenology* [Internet]. 2016;85(1):105-12. Available from: <http://dx.doi.org/10.1016/j.theriogenology.2015.07.019>
2. Al-Dossary AA, Caplan JL, Martin-Deleon PA. The contribution of exosomes/microvesicles to the sperm proteome. *Mol Reprod Dev* [Internet]. 2015;82:79-79. Available from: <http://doi.wiley.com/10.1002/mrd.22452>

P162 Progesterone regulates expression of mammal specific conserved miRNAs and their predicted targets in endometrial epithelial cells and may modify their secretion via extracellular vesicles in vitro

Hume Laura¹; Tinning Haidee¹; Wang Dapeng¹; Taylor Alysha¹; Simpson Nigel¹; Shillito Jayne²; O'Connell Mary³; Forde Niamh¹

¹University of Leeds; ²Leeds Teaching Hospitals NHS Trust; ³University of Nottingham

Hypothesis: We have identified a cohort of 14 microRNAs (miRNAs) that emerged at the origin of placental mammals and were never subsequently lost in any extant mammal lineage. We hypothesised that these conserved miRNAs, and the transcripts they regulate, play a critical role in modifying endometrial function to support early pregnancy success.

Materials and Methods: Ishikawa cells (n=3 biological replicates), were treated as follows: 1) control, 2) vehicle control 3) 0.1µg/mL progesterone (P4), 4) 1µg/mL P4, and 5) 10µg/mL P4 for 24 hr in the absence of serum. The expression of miRNAs and their regulated transcripts were assessed via miRNA PCR and single ended 75bp RNA sequencing (RNAseq) respectively. Extracellular vesicles (EVs) were isolated from the conditioned media via size exclusion chromatography and miRNA expression analysed. Differences in miRNA expression were determined via 2^{-ddct} method, and ANOVA while differentially expressed genes (DEGs) transcript expression was analysed via Rsubread and DESeq2 with an adjusted p<0.05.

Results: P4 decreased the expression of three miRNAs (mir671-5p, -542-3p and -340-5p) compared to controls (P<0.05). These miRNAs are predicted to regulate 16, 20, and 37 transcripts respectively associated with the gene ontology term "reproductive function". P4 treatment resulted in 391 DEGs (253 increased; 138 decreased). Of these DEGs, P4 treatment changed expression of 19 of the transcripts predicted to be regulated by these three P4-regulated miRNAs (P<0.05). Thirteen of the fourteen miRNAs were detected in EVs isolated from culture media however, P4 did not modify expression of these miRNAs in vitro.

Conclusions: Regulation of mir671-5p, -542-3p and -340-5p and their regulated transcripts by P4 indicates these may play a key regulatory role in establishing uterine receptivity to implantation. Moreover, detection of these miRNAs in extracellular vesicles secreted by the endometrial epithelium, indicates these miRNAs may regulate embryo development or decidualisation contributing to early pregnancy success.

P163 A set of microRNAs that emerged at the origin of placental mammals and retained thereafter, respond to physiological cues in the endometrium involved in early pregnancy success, in different species

Taylor Alysha¹; Tinning Haidee¹; Constantinides Bede¹; Orr David¹; Hume Laura¹; O'Connell Mary²; Forde Niamh¹

¹University of Leeds; ²University of Nottingham

Background: Mammal placenta emerged on the therian lineage ~180 million years ago and displays diverse morphology across mammal species. We have recently identified 14 miRNAs that emerged at the origin of placental mammals and were never subsequently lost in any extant mammal. We hypothesised that these miRNAs would be modified by key physiological cues required to establish successful early pregnancy in different mammal species.

Methods: Expression analysis of these 14 miRNAs was carried out on endometria tissue from bovine, porcine, murine and opossum and endometrial cells from humans. Bovine endometrial explants (bEXP), epithelial (bEECs), or stromal (bESCs) cells were treated with 1) control, 2) vehicle control, 3) recombinant ovine IFNT (roIFNT: 1ug/ml), or, 4) recombinant bovine CAPG (rbCAPG: 1 ug/ml). Human immortalized Ishikawa endometrial epithelial cells (hEECs) were treated with 1) control, 2) vehicle control, 3) Progesterone (P4:0.1ug/mL), 4) P4 (1ug/mL), 5) P4 (10ug/mL) or 6) rbCAPG. All experiments were carried out for 24 hr, with n=3 biological replicates. Significant differences in expression values were assessed using ANOVA when P<0.05.

Results: Expression of all 14 miRNAs was detected in all five species analysed. In human cells, miR-340-5p, miR-671-5p, and miR-542-3p expression decreased following treatment with the P4 (P<0.05). No effect on miRNA expression was observed in human cells treated with rbCAPG. In bovine miR-28-3p was decreased in bEXP treated with roIFNT (P<0.05). No effect of IFNT or CAPG was observed in bEECs however, in bESCs treated with 1ug/mL rbCAPG, expression of miR-340-5p and miR-433-3p decreased (P<0.05). Analyses of targets for these miRNAs identified a common target cyclooxygenase 15 (COX15).

Conclusions: Differential regulation of mammal specific miRNAs by physiological cues important for early pregnancy success indicate they may play a role in establishing successful pregnancy in species with different early pregnancy morphologies.

P164 Using a microfluidics approach to investigate how maternal metabolic stressors alter the proteomic composition of the uterine luminal fluid secretome produced in vitro

DeBem Tiago; Tinning Haidee; Moutevelidi Stefania; Vasconcelos Elton; Forde Niamh

University of Leeds

Hypothesis: We tested the hypothesis that physiological extremes of insulin and glucose modify the secretome of the uterus which may contribute to embryo loss/ dysfunction.

Materials and Methods: Primary endometrial epithelial and stromal cells were isolated from abattoir-derived early luteal phase bovine endometrium (n=6). Cells were cultured in RPMI (10% FCS; 1% ABAM). At ~80% confluency 6x10⁵ cells/mL of epithelial cells, and 1.5x10⁵ cells/mL of stromal cells were seeded in the upper and lower chamber of the microfluidics devices respectively (n=2 technical and n=3 biological replicates/treatment/experiment per) for 48 hrs. After a media change, cells were exposed to the following treatments for 72 hr (media flow rate of 1μL/min): Glucose 1) 0.5mM, 2) 5mM, and 3) 50 mM; Insulin 1) Vehicle, 2) 1ng/mL, and 3) 10ng/mL. The upper chamber media was collected, spun at 400xg for 10 min at 4°C, and snap frozen. Quantitative differences in proteins between groups were identified using Tandem Mass Tag (TMT) proteomics. Fold change differences in protein abundances between groups was determined using paired t-tests and were considered significant when p < 0.05.

Results: The abundance of a similar number of proteins were modified in the upper chamber media following exposure of cells to 1ng (73 proteins: 10 increased, 63 decreased), 10ng (76 proteins: 1 increased, 75 decreased), or 10ng v 1ng (67: 5 increased, 62 decreased) of insulin. In contrast, glucose only altered the abundance of 8 (50 μ m v 0.5 μ m glucose) or 21 (50 μ m v 5 μ m) proteins respectively. There was no overlap in the proteins that were modified by glucose while a small proportion (3 proteins) were modified in all three insulin treatment comparisons.

Conclusion: In conclusion, concentrations of insulin rather than glucose contribute the most to altering the uterine endometrial secretome during the early luteal phase.

P165 Microfluidic devices can isolate higher quality sperm compared to density gradient centrifugation

Kaewman Paweena¹; Meissner Max²; Thanoi Samur¹; Nudmamud -Thanoi Sutisa¹; Reynolds Steven³; Kantsler Vasily²; Pacey Allan³

¹Naresuan University, Thailand; ²University of Warwick, UK; ³University of Sheffield, UK

Background: The recovery of high-quality sperm from semen is essential for assisted reproductive technologies such as IVF and ICSI. Whilst Density Gradient Centrifugation (DGC) is an established technique, it requires centrifugal forces which can damage sperm. We evaluated a novel microfluidic device based on asymmetric micro structured barriers as an alternative sperm separation method to DGC.

Methods: Sperm were recovered from 14 ejaculates (n=9 normal and n=5 abnormal by World Health Organisation criteria) using a standard DGC and the novel microfluidic device. Measures of sperm concentration, progressive motility, percent motility, normal sperm morphology and sperm DNA fragmentation (using the TUNEL) assay were evaluated by both methods and in the original semen sample. All data shown is mean $\pm$ SEM.

Results: Compared to DGC, the sperm selected by microfluidics had significantly higher progressive motility ($83.1 \pm 2.9\%$ vs $65.2 \pm 4.3\%$ in normal ejaculates and $72.8 \pm 2.5\%$ vs $44.0 \pm 2.4\%$ in the abnormal ejaculates), percent motility ($94.6 \pm 1.4\%$ vs $83.9 \pm 2.4\%$ in normal ejaculates and $88.1 \pm 2.8\%$ vs $66.1 \pm 3.6\%$ in the abnormal ejaculates) as well as an increase in sperm morphology ($10.5 \pm 2.2\%$ vs $6.2 \pm 1.3\%$ in normal ejaculates and $9.5 \pm 3.3\%$ vs $7.0 \pm 2.3\%$ in the abnormal ejaculates). Notably, the percent of sperm with fragmented DNA was significantly lower after recovery from the microfluidic devices than those selected by DGC ($0.7 \pm 0.2\%$ vs $7.3 \pm 1.1\%$ in normal ejaculates and $1.0 \pm 0.1\%$ vs $9.8 \pm 1.1\%$ in the abnormal ejaculates).

Conclusions: The novel microfluidic device can select sperm of high quality and low levels of DNA fragmentation from both normal and abnormal ejaculates without the need for centrifugation.

P166 Examining the impact of mitosis duration on embryo development

Allais Adélaïde; FitzHarris Greg

CRCHUM, Université de Montréal, Canada

Background: The timing of cell divisions is highly variable in mammalian embryos, and is a potential indicator of embryo health in fertility clinics (termed morphokinetics). In somatic cells, extended mitosis can cause premature separation of sister chromatids, a phenomenon known as "cohesion fatigue" (CF), which could cause aneuploidy. Separately, a so-called mitotic clock checkpoint (MitClock) has been recently described wherein an extended duration of mitosis can cause a subsequent G1/S arrest. We set out to determine whether cohesion fatigue can occur, and whether the MitClock operates, in mouse preimplantation embryos.

Methods: We manipulated the duration of mitosis in two-cell stage mice embryos with the anaphase promoting complex inhibitor APCin. Live and fixed confocal imaging of the spindle, kinetochores, and DNA were performed.

Results: Mitosis prolongation causes an increase of spindle length and a time-dependent loss of chromosome alignment. 4% of all sister pairs had individualized by 6 hours of mitotic arrest, and 66% by 24 hours. The loss of chromatid cohesion was preceded by an increase in inter-kinetochore distances from 0.59 μm to 0.78 μm ($p < 0.0001$), consistent with CF. 24 hours mitosis prolongation in the presence of a spindle poison did not trigger CF, suggesting it is spindle-tension-dependent. Strikingly, live imaging revealed that 6 hours mitosis prolongation does not prevent subsequent embryo development, but doubled the frequency of micronuclei per embryo (4.5 vs 2.7), suggesting that CF leads to aneuploidy. In contrast, 24 hours mitotic arrest causes a potent cell cycle arrest in the subsequent interphase. Preliminary data suggests that this arrest may be dependent upon a critical level of cohesion fatigue.

Conclusion: Moderately prolonged mitoses fail to activate a mitotic clock checkpoint in embryos, but lead to appreciable increases in cohesion fatigue and chromosome segregation defects. Our data allude that cohesion fatigue could be a previously unappreciated cause of mosaic aneuploidy in the mammalian embryo.

P167 Placental endocrine malfunction programs metabolic derangements in offspring

Rodgers Amanda; Yong Hannah; Lopez-Tello Jorge; Sandovici Ionel; Constanica Miguel; Sferruzzi-Perri Amanda

University of Cambridge

Introduction: Studies have shown that a mother's environment (diet, stress and oxygen levels) can affect placental growth, birthweight, and program the offspring's long-term health. However, the precise role of the placenta, the site of materno-fetal nutrient transfer and source of metabolism-modulating hormones, in developmental programming of the offspring is unknown. Utilizing a mouse model where placental endocrine malfunction was selectively induced by loss of the imprinted insulin-like growth factor-2 gene in the endocrine zone of the placenta (Jz- ΔIgf2), this project aimed to identify the role of placental endocrine function in the metabolic health of offspring.

Methods: TpbpaCre females were crossed with Igf2-floxed males to produce entire litters with Jz- ΔIgf2 . Litters of the reverse parental cross (no change in Igf2) were used as controls. After birth, litters were standardised to 3 females and 3 males and, from weaning, pups were fed a normal chow or high-fat, high-sugar (HFHS) diet (>6 litters/genotype/diet). Insulin tolerance tests were performed at 12 weeks of age and metabolic tissues collected at 13 weeks for molecular and biochemical analyses. T-tests were used to determine significant differences between Jz- ΔIgf2 and controls within each diet and sex ($P < 0.05$).

Results: Regardless of diet and sex, Jz- ΔIgf2 mice were significantly insulin resistant compared to controls. On a chow diet, female Jz- ΔIgf2 offspring had reduced adiposity, greater pancreatic insulin and altered hepatic glycogen and lipid content compared to controls. However, there was no difference in pancreatic insulin, liver composition or adiposity between Jz- ΔIgf2 males and controls fed a chow diet. On a HFHS diet, Jz- ΔIgf2 males, but not females, had increased adiposity compared to controls. In the liver, there were differences in the abundance of growth and metabolic signalling proteins between Jz- ΔIgf2 and control offspring dependant on diet and sex.

Conclusion: Placental endocrine malfunction (via Jz- ΔIgf2) impacts the metabolic health of offspring.

P168 Developmental programming of Mesenchymal Stem/Stromal cells (MSCs) by foetal growth restriction

Esteves Cristina; Avikina Viktorija; Klein Blake; Cortes-Araya Yennifer; Dan-Jumbo Susan; Stenhouse Claire; Donadeu Xavier

The Roslin Institute

Background: Foetal growth restriction (FGR) affects 10-40% new born babies. It has a multifactorial origin and often results from inadequate placental function. FGR offspring have low birthweight and altered postnatal development including a predisposition to excessive body fat accumulation. They are also at high risk of metabolic (type II diabetes, cardiovascular) and other diseases later during life. IUGR occurs spontaneously in multiparous species such as the pig

where it shows features that recapitulate human IUGR. The objective of this study was to investigate the effects of IUGR on mesenchymal progenitor cell properties using a porcine model.

Methods: MSCs were obtained by enzymatic digestion of porcine subcutaneous adipose tissue collected from FGR (defined as birthweight <70% the litter average) and normal littermates at birth (n=6), and their growth and differentiation capacities in culture were compared.

Results: MSCs from both FGR and normal piglets grew at similar rates, clonally and expressed typical MSC markers (CD105, CD90, CD44). However, differentiation capacity of MSCs was significantly altered by FGR. Upon induction, adipogenesis was more pronounced in FGR than normal MSCs, as indicated by higher Oil Red O staining and expression of FABP4 and PPARG. Conversely, both chondrogenic and osteogenic capacities were decreased in FGR MSCs as indicated by a reduce size of chondrogenic pellets which had reduced expression of SOX9, and increased staining for Alizarin Red after osteogenic differentiation, respectively. Interestingly, FGR MSCs had higher fibrotic potential as indicated by higher Collagen 1 expression upon induction of fibrosis. These results are consistent with the observation in vivo than FGR offspring develop higher body fat content and tissue fibrosis at the expense of other mesenchymal lineages. These findings also suggested that IUGR MSCs are programmed in the uterus what may underpin an increased disease risk in FGR humans, including hypertension and diabetes during adult life.

P169 Can monitoring progesterone levels in frozen embryo transfer cycles reduce pregnancy loss

Lodge Yvonne; Louisy Andrina; Baranowski Diana; Reid Judy; Campbell Alison

CARE Fertility, Tunbridge Wells

Introduction: The efficacy of luteal support has been reviewed in recent years with no conclusion as to best practice (Cochrane Review, 2010). Levels of serum progesterone vary depending on the route of administration (Shapiro et al., 2014). However there is no consensus on the ideal protocol or level to maximise outcome.

Materials and Methods: 256 FET cycles were retrospectively analysed and compared with 135 FET cycles where patients had a progesterone blood test prior to or on the day of embryo transfer. Patients with blood results showing <50 nmol/L progesterone were offered additional progesterone support. Outcomes were measured by positive pregnancy test and clinical pregnancy confirmed by ultrasound scan at 7 weeks gestation.

Results: In the <38 age group the biochemical loss (BL) in patients who did not have progesterone testing was 28% (28/99) and reduced to 14% (8/58) (p=0.00258) in the group where progesterone levels were taken. In ≥ 38 age group the BL in non-tested patients was 44% (11/24) and reduced to 21% (4/19) (p=0.0004) in the tested group. Serum progesterone levels averaged at 35.4 nmol/L and varied from 7.4 to 179 nmol/L in patients following the same initial drug regime. Of the patients tested that did not require additional support the pregnancy rate was 40% (18/45) with a clinical pregnancy rate (CPR) of 37% (17/45). This resulted in a biochemical pregnancy loss of 8%. Where additional support was given the pregnancy rate was 61% (55/90) with a CPR of 49% (44/90). The biochemical pregnancy loss was 20%.

Conclusion: Progesterone absorption between patients and although measuring progesterone levels does not appear to reduce biochemical pregnancy loss in all patients, it seems to be helpful in patient's ≥ 38 years old. Therefore tailoring of the individuals' treatment can lead to increased implantation and ongoing clinical pregnancy rates.

Glujovsky D, Pesce R, Fizbajn G, Sueldo C, Hart RJ, Ciapponi A. Endometrial preparation for women undergoing embryo transfer with frozen embryos or embryos derived from donor oocytes. Cochrane Database Syst Rev 2010. Sapiro D, Boostanfar R, Silverberg K, Yanushpolsky, EH. Examining the evidence: progesterone supplementation during fresh and frozen embryo transfer Reproductive BioMedicine Online 2014: 29, S4-S14.

P170 Pressure effects of oil and water-soluble contrast media in tubal flushing: A pilot study

Hajiyavand Amir M¹; Kothandaraman Anjana¹; Roest Inez²; Koks Carolien²; Mijatovic Velja³; Mol Ben W⁴; Dearn Karl D¹

¹University of Birmingham; ²Maxima Medical Centre; ³Amsterdam University Medical Centre; ⁴University of Monash

Introduction: Tubal flushing during Hysterosalpingography (HSG) with an Oil-Soluble Contrast Medium (OSCM) results in a higher rate of ongoing pregnancies compared to Water-Soluble Contrast Medium (WSCM) (39.7% vs 29.1%)[1-3]. The mechanisms behind this effect are still unclear.

Objectives: This study investigates a method to determine variations in the build-up of pressure within the fallopian tube during HSGs using OSCM and WSCM.

Method: We designed an in-vitro experimental setup that incorporated a 3D printed fallopian tube utilising dimensions available from anatomical studies. The synthetic fallopian tube, including artificial occlusion sites, is connected to a syringe pump which introduces 9mL, including 0.7mL dynamic flow volume of the contrast at a constant injection rate of 24.5mL/min. A pressure sensor measures the pressure build-up at the site of occlusion. This set up mimics the HSG procedure using controlled injection parameters. The employed tube is manufactured to be fully rigid without any compliance, so that the fluid power effect are isolated.

Results: Preliminary results from 30 in-vitro experiments indicate that the use of OSCM during HSG results in a higher build-up of pressure within the tube compared to WSCM (20.56 ± 11.96 kPa). The pressure values for OSCM and WSCM with full occlusion were 163.59 ± 11.22 kPa and 143.69 ± 2.45 kPa, respectively. Higher fluidic power may assist in dislodging debris/mucus from the internal wall of the fallopian tube and flush it towards the abdominal cavity, facilitating tubal patency in otherwise anatomically normal fallopian tubes.

Conclusion: Results from 30 in-vitro experiments indicate that using OSCM during HSG induces 14.3% (± 5.8 SD) higher fluidic power compared to WSCM. This differential may contribute to the dislodging of debris and mucus in the proximal part of the tube. This pressure build-up is the result of the physical properties such as viscosity of the oil-based compared to Water-based contrasts (reported as 0.034-0.07Pas and 0.0027-0.0143 Pas Respectively).

1. Dreyer, K., Van Rijswijk, J., Mijatovic, V., Goddijn, M., Verhoeve, H.R., Van Rooij, I.A., Hoek, A., Bourdrez, P., Nap, A.W., Rijnsaardt-Lukassen, H.G. and Timmerman, C.C., 2017. Oil-based or water-based contrast for hysterosalpingography in infertile women. *New England Journal of Medicine*, 376(21), pp.2043-2052. 2. Wang, R., van Welie, N., van Rijswijk, J., Johnson, N.P., Norman, R.J., Dreyer, K., Mijatovic, V. and Mol, B.W., 2019. The effectiveness of tubal flushing with different contrast media on fertility outcomes: a systematic review and network meta-analysis. *Ultrasound in Obstetrics & Gynecology*. 3. Fang, F., Bai, Y., Zhang, Y. and Faramand, A., 2018. Oil-based versus water-based contrast for hysterosalpingography in infertile women: a systematic review and meta-analysis of randomized controlled trials. *Fertility and sterility*, 110(1), pp.153-160.

P171 Insulin-like growth factor 2 overexpression alters murine placental structure

Aykroyd Bethany; Tunster Simon; Sferruzzi-Perri Amanda

Centre for Trophoblast Research, Department of Physiology, Development and Neuroscience, University of Cambridge, Cambridge, UK

Background: Abnormal fetal growth can have immediate and life-long consequences for offspring health. The placenta regulates fetal nutrient allocation and secretes hormones which adapt maternal metabolism to support fetal growth during pregnancy^[1]. Insulin-like growth factor 2 (Igf2) is a paternally-expressed imprinted gene. In mice, global overexpression of Igf2, driven by deletion of the reciprocally imprinted H19 gene, leads to fetoplacental overgrowth and an increase in placental endocrine zone (Jz) formation and glycogen storage^[2,3]. However, the specific role of Igf2 within the Jz in regulating placental endocrine function is unknown. Moreover, the contribution of fetal sex in determining fetoplacental outcomes with Igf2 manipulation is unexplored. This study aimed to determine the effect of overexpressing Igf2 in the Jz (Jz-Igf2OE) on placental endocrine phenotype in both fetal sexes.

Methods: Heterozygous H19Flox females were mated with homozygous TpbpaCre male mice to generate litters of mixed genotype; 50:50 control and Jz-Igf2OE. Fetal and placental weights were measured on day 16 of pregnancy (term ~day 20.5) when the Jz is largest and when the mouse dam is most insulin resistant and glucose intolerant. Conceptuses were sexed through Sry genotyping and the effect of Jz-Igf2OE on placental structure was assessed using stereology and glycogen assays. Significant genotypic differences within each sex were assessed by t-test ($P < 0.05$).

Results: Fetal and placental weights were unaltered with Jz-Igf2OE. However, the volume of Jz spongiotrophoblasts were increased in both sexes. Moreover, in females, the volume of Jz glycogen and trophoblast giant cells were increased. Placental glycogen concentrations were also significantly increased in Jz-Igf2OE females only.

Conclusions: Jz-Igf2OE alters the cellular composition of the placental Jz. However, these changes were dependent on fetal sex. Work is underway to assess the impact of Jz-Igf2OE on the size of placental glycogen cells and expression of IGF2 receptors, hormones and endocrine cell markers.

1. Napso T, Yong H, Lopez-Tello J, Sferruzzi-Perri AN. The role of placental hormones in mediating maternal adaptations to support pregnancy and lactation. *Frontiers in Physiology*. 2018;9:1091.
2. Angiolini E, Coan PM, Sandovici I, Iwajomo OH, Peck G, Burton GJ, Sibley CP, Reik W, Fowden AL, Constância M. Developmental adaptations to increased fetal nutrient demand in mouse genetic models of Igf2-mediated overgrowth. *The FASEB Journal*. 2011;25(5):1737-45.
3. Esquiliano DR, Guo W, Liang L, Dikkes P, Lopez MF. Placental glycogen stores are increased in mice with H19 null mutations but not in those with insulin or IGF type 1 receptor mutations. *Placenta*. 2009;30(8):693-9.

P172 Oocyte cytoplasmic maturation: design, organisation and synchrony

Vinals Gonzalez Xavier¹; Thrasivoulou Christopher²; Gaunt Matthew¹; Seshadri Srividya¹; Serhal Paul¹; Sen Gupta Sioban³

¹The Centre for Reproductive and Genetic Health; ²Research Department of Cell and Developmental Biology, University College London; ³Institute for Women's Health, University College London

Background: Defining oocyte competence remains to be one of the goals of contemporary reproductive science. Morphology-based description has proven to be inconsistently associated with development and clinical outcomes, which has led to little to no consensus from the scientific community. Oocyte maturation not only involves correct chromosome segregation and positioning but also cytoplasmic remodelling events. While nuclear maturation has been well characterised, ooplasmic maturation and its clinical consequences continue to be ill defined.

Aims: This pilot study aims to describe novel non-invasive imaging parameters associated with the nature of the ooplasm along the different maturation stages.

Methods: This study assessed 290 human oocytes (37 GVs, 31 MIs and 222 MIIs) retrieved 37 hours post trigger and further denuded to assess nuclear maturity. Light microscopy was conducted with an Olympus IX53 microscope including a Nikon D300 camera. All images were processed with ImageJ for their analysis.

Results: Mean optical density (OD, $\pm$ SD) for the three different stages of oocyte maturation was: 0.167 ± 0.094 (GV), 0.072 ± 0.061 (MI) and 0.046 ± 0.045 (MII). Statistical analysis for differences in mean in the different groups showed significance when comparing GV to MI ($p < 0.001$) and GV to MII ($p < 0.001$). No difference was reported between MI and MII groups ($p = 1.00$). Within the ooplasm, OD distribution along the different stages shows central vs peripheral differences. Gray level co-occurrence matrix (GLCM) were used to analyse texture parameters, revealing differences in linear dependency, homogeneity and local variations in the ooplasm of GV, MI and MII-stage oocytes.

Conclusions: GV-stage is characterised by higher OD which tends to be centrally located. Moreover, OD distribution and texture reflects the dynamic nature of the ooplasm. OD variations and/or specific texture patterns could be related to failure or poor cytoplasmic re-organisation, compromising the competence of the female gamete prior to insemination.

P173 Determining true background rate of endometriosis and if diagnosis preoperatively can be made, in a low risk population, utilising a questionnaire

Tempest Nicola¹; Whelan Amy²; Phelan Marie²; Hapangama Dharani¹

¹Liverpool Women's Hospital/University of Liverpool; ²University of Liverpool

Background: Endometriosis is an oestrogen dependent chronic inflammatory condition thought to affect approximately 1 in 10 women of the general population and up to 1 in 2 patients presenting with infertility. Endometriosis is poorly understood and requires a diagnostic laparoscopy to diagnose. Laparoscopic sterilisation is an operation for permanent contraception.

Objectives: Determine background endometriosis rates in a truly control population. Diagnose endometriosis with a simple questionnaire prior to surgery.

Methods: Women undergoing laparoscopic sterilisation (no previous pelvic surgery) were recruited following written consent. Patients completed a questionnaire and surgeon completed proforma documenting presence/absence of endometriosis, stage, adhesions.

Results: 102 women underwent laparoscopic sterilisation, mean age 36 years, mean BMI 27.5. 12 women were incidentally diagnosed with endometriosis (11.8%), 9 (8.8%) stage I, (2%) stage II, 1 (1%) unknown. Comparing endometriosis (12) to non-endometriosis (90) women, employing their pre-surgical questionnaire, 4 (33.3%) with endometriosis diagnosis complained of pain all of the time during their period versus 28 (31.1%) of the non-endometriosis patients. 3 (25%) of the endometriosis women had pain all of the time before their period versus 26 (28.9%) of the non-endometriosis women. 6 (50%) diagnosed with endometriosis complained of heavy periods all of the time versus 17 (18.9%) of the non-endometriosis women. 3 (25%) diagnosed with endometriosis had prolonged periods all of the time versus 7 (7.8%) of the non-endometriosis women. 3 (25%) diagnosed with endometriosis had problems conceiving versus 8 (8.9%) of the non-endometriosis women.

Conclusions: Our 'incidental' findings of endometriosis are slightly higher than the quoted background incidence. Women with endometriosis seem to have longer and heavier periods. Interestingly, a large proportion of our cohort complained of pelvic pain despite attending for laparoscopic sterilisation. More research needs to be completed as non-invasive diagnosis would aid many women and medical professionals to treat/reassure women in a timely fashion.

P174 Using nuclear magnetic resonance metabolomics as a non-invasive way of diagnosing endometriosis

Tempest Nicola¹; Whelan Amy²; Phelan Marie²; Hapangama Dharani¹

¹University of Liverpool/Liverpool Women's Hospital; ²University of Liverpool

Background: Endometriosis is an oestrogen dependent chronic inflammatory condition affecting 1 in 10 women of the general population and 1 in 2 with infertility. Endometriosis is poorly understood and diagnosis is through an invasive laparoscopy. Metabolomics is in-depth study and quantitative analysis of small molecules found in cells, tissues and body fluids. As pathology can alter metabolic composition of biofluids, nuclear magnetic resonance (NMR) can gather this data and reveal important findings from complex diseases.

Objectives: Use NMR to generate spectra and quantify key metabolites found in serum of women with endometriosis.

Methods: Blood was collected preoperatively from women with surgically diagnosed endometriosis/ no endometriosis and 1D 1H-NMR was acquired at 37°C on a 600 MHz (bruker-avanceIII TCI-cryoprobe). Relative metabolite abundances underwent statistical analysis using MetaboAnalyst 3.0 (p-values FDR adjusted).

Results: 133 women mean age 36.6 years, BMI 27.4 and 48 (36.1%) smokers were recruited. Endometriosis was diagnosed in 26 (19.5%) of the women, 11 (8.3%) stage 1 and 2 and 15 (11.3%) stage 4. One metabolite (identity unknown) could differentiate between women with and without endometriosis ($p < 0.01$). Additional metabolites including 3-hydroxybutyrate could differentiate between endometriosis and no endometriosis ($p < 0.01$) although not when smokers and /or obesity were filtered. When stage 4 endometriosis were compared to no endometriosis, numerous metabolites including Lactate and 3-hydroxybutyrate could differentiate between the groups ($p < 0.01$).

Conclusions: Endometriosis and non-endometriosis patients can be differentiated between using NMR metabolomics on serum. Previous studies using the same technique in the hopes of finding a non-invasive biomarker to aid a more accessible and rapid diagnosis of endometriosis, have not been validated and study populations applicable to the general population were not utilised. These preliminary results can be utilised on a test cohort to examine if reproducible robust models can be created leading to a non-invasive diagnostic test for endometriosis.

P175 Bisphenol S causes mitochondrial dysfunction and meiotic defects during pig oocytes maturation

Prokesova Sarka¹; Zalmanova Tereza¹; Hoskova Kristyna¹; Benc Michal¹; Nevoral Jan²; Petr Jaroslav¹

¹Institute of Animal Science, Prague; ²Biomedical Center, Faculty of Medicine, Charles University, Pilsen, Czech Republic

Background: Endocrine disruptors (EDs) are substances in the environment, disturbing the balance of the body's physiological functions at the hormonal control level. EDs can affect reproductive functions by reducing fertility at very low doses[1]. Demonstration of the endocrine disruptive effect of the Bisphenol A leads to its replacement by bisphenol S (BPS). However last year's studies have declared that it is not the best alternative[2]. One of the possible BPS action mechanism is the generation of the oxidative stress and thus affection of oocyte quality through modulation of mitochondrial functions. Dysfunctional mitochondria significantly decrease ATP synthesis in oocytes, with adverse effects on spindle formation and chromosomal segregation resulting in decreased oocyte quality and aneuploidy[3,4]. The aim of the study was to examine changes in the organization of mitochondria, spindle microtubules and aneuploidy occurrence during meiotic maturation of porcine oocytes exposed to very low doses of BPS in vitro.

Methods: Porcine ovaries were collected from pre-pubertal gilts at a local slaughterhouse. Fully grown germinal vesicle (GV) stage oocytes (N=150) were subjected to in vitro maturation (IVM) in presence of 3 different concentrations of BPS (3 μ M, 30 nM, 300 pM). To assess the mitochondrial reorganization and the developmental potential (cytoskeletal changes, chromosomal aneuploidy rating), immunofluorescence staining (MitoTracker, anti α tubulin, CREST) was performed.

Results: The BPS treated MII oocytes exhibited aberrant mitochondrial reorganization and faultless organization of tubulin filaments and chromosomes in the spindle apparatus compared to the control group. Furthermore, an increased incidence of chromosomal aneuploidy has been demonstrated, indicating BPS as aneuploidy-inducing agents

Conclusion: The presence of BPS during oocyte meiotic maturation results in mitochondrial organization changes, spindle misalignment and increased incidence of chromosomal aneuploidy, which might contribute to the poor developmental potential of embryos. The presented results are inevitable to clarify the mechanism by which BPS influence mammalian reproduction.

1 Sifakis S, Androutsopoulos VP, Tsatsakis AM, Spandidos DA. 2017. Human exposure to endocrine disrupting chemicals: effects on the male and female reproductive systems. *Environ Toxicol Pharmacol*, 51: 56-70. doi: 10.1016/j.etap.2017.02.024 2 Eladak S, Grisin T, Moison D, Guerquin MJ, Tumba-Byn T, Pozzi-Gaudin S, Benachi A, Livera G, Rouiller-Fabre V, Habert R. 2015. A new chapter in the bisphenol A story: bisphenol S and bisphenol F are not safe alternatives to this compound. *Fertility and sterility*, 103: 11-21. doi: 10.1016/j.fertnstert.2014.11.005 3 Van Blerkom J. 2004. Mitochondria in human oogenesis and preimplantation embryogenesis: Engines of metabolism, ionic regulation and developmental competence. *Reproduction*, 128: 269-80. doi: 10.1530/rep.1.00240 4 Ramalho-Santos J, Varum S, Amaral S, Mota PC, Sousa AP, Amaral A. 2009. Mitochondrial functionality in reproduction: From gonads and gametes to embryos and embryonic stem cells. *Hum Reprod Update*, 15: 553-72. doi: 10.1093/humupd/dmp016

P176 In an egg donation programme, does AMH, age and history of previous pregnancy predict the number of mature oocytes obtained?

Pataia Vanessa; Wolska Marta; Wang Jinjun; Macklon Nick; Ahuja Kamal

London Egg Bank, London Women's Clinic

Background: A relationship between age, Anti-Mullerian hormone (AMH) and ovarian stimulation response is well established in the subfertile population. However, the value of these markers in healthy egg donors remains to be clarified.

Objectives: To evaluate the impact of donor age, AMH and history of previous pregnancy on ovarian response to stimulation assessed by total number of oocytes and total number of mature oocytes collected.

Methods: Retrospective cohort analysis of 223 egg donors aged 18-35 registered in accordance with the Human Fertilisation and Embryology Authority (HFEA) guidance, and divided into 6 age groups: 18-20 (n=28), 21-23 (n=60), 24-26 (n=39), 27-29 (n=45), 30-32 (n=27), 33-35 (n=24). Kruskal-Wallis test was applied to compare the relationship between study parameters and outcomes between age groups. The respective associations within each age group were investigated using Spearman's rank correlation. Mann-Whitney U test was performed to assess the effect of previous pregnancies on study parameters and outcomes.

Results: No significant differences were obtained in mean AMH levels, total oocytes or total mature oocytes between age groups. Moreover, a history of previous pregnancy had no significant impact on study parameters. AMH was

however positively correlated with the number of mature oocytes obtained, and the strength of association increased with age. Similar findings applied to the total number of oocytes obtained, particularly in donors ≥ 27 years-old.

Conclusions: In a cohort of healthy egg donors up to 35 years-old with normal ovarian reserve, neither AMH levels or the number of total/mature oocytes varied with age or history of previous pregnancy. However, AMH was predictive of the number of total/mature oocytes obtained, and the strength of this association increased with age. These findings suggest that donors meeting standard ovarian reserve criteria for acceptance produce similar numbers of mature oocytes irrespective of age or history of previous pregnancy.

P177 Is the presence of multi-nucleation on Day2/3 associated with reduced implantation potential?

Payne Ashley; Zujovic Lyndsey

Nurture Fertility

Background: Time-lapse monitoring (TLM) has introduced a wealth of new parameters we can monitor in embryo development compared to traditional microscopic examination. There is expanding literature discussing the clinical relevance of these 'new parameters' with the aim of establishing if the data available through TLM can improve embryo selection and subsequent clinical outcome. One of the areas of interest is the appearance of multi-nucleation at the cleavage stage. Observations of multi-nucleation (MN) are not exclusive to TLM, however the likelihood of noting MN during traditional culture is greatly reduced. The aim of this study was to determine whether the presence of MN affects the embryos implantation potential.

Method: 642 embryos with known implantation outcome were retrospectively analysed for the presence or absence of MN on Day 2 and 3 of development. A total of 402 fresh embryo transfers were performed on Day 5 of development between 1/7/2017-1/7/2019. All embryos were cultured in Vitrolife GTL in an Embryoscope plus incubator.

Results: MN was observed in 25.9% (166/642) of embryos transferred. The implantation rate per embryo transferred was 40.5% (193/476) following the transfer of non-MN embryos versus 27.1% (45/166) for patients who had MN embryos transferred ($p=0.0021$). The clinical pregnancy rate per embryo transfer was 50% (158/316) in patients who only had non-MN embryos transferred versus 37.2% (32/86) in patients who had only MN embryos transferred ($p=0.0387$). Other factors which may influence outcome were also analysed, namely; patient age, AMH, average number of oocytes collected and number of attempts.

Conclusion: Embryos with MN at the cleavage stage have a significantly reduced implantation potential. MN should therefore be considered as part of future embryo transfer selection criteria.

P178 The pig as a model for cloacal malformations

Scriven Lucy¹; Gardner David¹; Wood Richard²; Sutcliffe Jonathan³; Williams Alun⁴

¹University of Nottingham; ²Nationwide Children's Hospital, Columbus, Ohio, USA; ³Leeds General Infirmary; ⁴Nottingham University Hospitals NHS Trust

Aim of Study: Investigation into the surgical management of cloaca malformations requires an animal model, female pigs have a single urogenital opening. We aimed to assess and evaluate a model of urogenital sinus using juvenile pigs aged 7 - 12 weeks (10 - 30kg body mass).

Methods:

The urogenital tracts of juvenile pigs (3 per time point) aged 7 - 12 weeks were characterised thus:

- Resin casting to establish dimension and shape of both the urogenital tract and surrounding connective tissue and vasculature.
- Histology (H&E and picrosirius red)
- Immunohistochemistry: Ki67 (for characterising proliferative cells), Vimentin (mesenchymal cells), E-Cadherin

(epithelial cells), S-100 (neurons) and α -SMA (vascular and other smooth muscle).

Porcine tissue was compared with published hysterectomy histology, radiological studies and paediatric histology of human urogenital tract.

Results: The resin cast of the juvenile porcine urogenital tract showed the vagina and urethra open into a common channel, the urogenital sinus, this included a hymenal narrowing between the vagina and urogenital sinus. The vascular cast showed a mesentery-like meshwork of vessels surrounding the vagina and large branches from the uterine and vaginal arteries, with multiple anastomoses supplying the urogenital tract. Histologically, pubescent porcine tissue showed a comparable morphology with vaginal hysterectomy tissue. Juvenile tissue had a single cell columnar epithelium, with the underlying tissue having a homogenous, mesenchymal appearance, this result was comparable to the paediatric histology. The juvenile pig had higher levels of positive staining of Ki67 and Vimentin, when compared to the pubescent porcine tissue.

Conclusion: The urogenital tract of the juvenile pig at 9-10 weeks has broadly comparable histology to human. The histology, morphology, vasculature and neural supply of the porcine urogenital tract support the assertion that it is likely to be responsive to expansion, which would facilitate surgical techniques for repair of cloacal

P179 Deep learning assessment of ICSI morphology as a predictor of ICSI success

Colangelo Angela¹; He Peter¹; Toschi Marco²; Lavery Stuart¹; Hickman Cristina¹

¹Imperial College London; ²Instituto Valenciano De Infertilidad SA

Objective: The impact that oocyte morphology has on the outcome of ICSI procedures has been analysed in numerous studies. However, despite years of research, the evidence is still contradictory as to whether oocyte morphology has an impact on fertilisation outcome or is influenced more by other factors. The purpose of this study was to apply a supervised Machine Learning (ML) model to oocyte images prior to ICSI to determine whether we can predict normal fertilisation (2PN), over-fertilisation (+3PN), non-fertilisation and degeneration based on oocyte morphology.

Design: Retrospective analysis performed on images from anonymised videos of ICSI procedures. Material and

Methods: The oocyte images were analysed using two different supervised ML models. The first model was a detection model implemented from YOLOv3 aimed to detect and localise oocytes in microscopy images. The second model was a classification model, implemented from the Inception-v3 deep learning (DL) model. The aim of the classification model was to classify fertilisation outcomes based on oocyte morphology prior to ICSI. The models' performances were evaluated using Average Precision (AP), Intersection over Union (IoU), specificity and sensitivity metrics. The accuracy of the classification model was also calculated.

Results: The detection model was able to detect oocytes from the captured images (AP 100%, IoU 91.4%, sensitivity 0.95). The classification model was not able to classify oocyte fertilisation outcomes based on oocyte morphology (for 2PN classification: 0.76 sensitivity, 0.83 specificity; for nonfertilised and degenerate classification: 0.61 sensitivity, 0.5 specificity). The overall model accuracy was 0.5.

Conclusions: This study suggests that oocyte morphology is not a good predictor of fertilisation and ICSI success.

P180 The operator during oocyte retrieval matters: Findings from a large cohort of more than 10,000 cycles

Papathanasiou Thanos; Prasannan-Nair Chhaya; Babbur Vijayalakshmi; Mauro Valentina; Hapuarachi Sharleen; Mawal Nausheen

Bourn Hall Clinic

Background: It has been suggested that the operator is influential to the oocyte retrieval (OCR) yield (1). The aim was to compare the harvesting ability of OCR operators with different levels of experience in the largest cohort of patients to-date.

Methods: Consecutive cycles that led to OCR between 2006—2018 were included. The OCRs were performed by 7 operators (2 very experienced with more than 3,000 OCRs each, 3 experienced with 500-1,500 OCRs each, 2 less experienced with 100-500 OCRs each).

Multivariable analysis was performed between the 'operator' and the 'number of retrieved (and MII) oocyte' variables, adjusting for confounders known to influence the oocyte yield (age, BMI, agonist or antagonist protocol, number of recruited follicles on trigger day).

Generalised estimating equations (binomial with a log link) were used, as they provide a good fit for the positively skewed oocyte variable and also account for correlated cycles. Bonferroni adjustment was applied for multiple comparisons.

Results: A total of 10,445 cycles with retrieved oocytes and 6,368 ICSI cycles with MII oocytes were analysed.

There was significant difference in the oocyte yield between the highest and lowest retrieving operator (mean difference 2.2 95%CI 0.8-3.7 retrieved oocytes, 1.5 95%CI 0.1-3.0 MII oocytes).

The highest mean oocyte numbers were retrieved by a very experienced (10.1 oocytes) and a less experienced operator (9.5 oocytes), while the lowest numbers were retrieved by two experienced operators (7.9 and 8.4 oocytes) and a less experienced operator (7.9 oocytes).

Conclusions: There is significant variation in performance between operators during oocyte retrieval. However, as long as the operator has performed a reasonable number of procedures (>100 OCRs), experience does not appear to be the critical factor influencing performance. Other factors, such as training or personal acuity, may be more relevant.

Future IVF research should account for the OCR operator.

1. Rustamov O, Wilkinson J, La Marca A, Fitzgerald C, Roberts SA. How much variation in oocyte yield after controlled ovarian stimulation can be explained? A multilevel modelling study. *Hum Reprod Open* [Internet]. 2017 Nov 1 [cited 2018 Dec 3];2017(3).

P181 Involvement of ATP in the differential sensitivity of InsP3- and Sr2+- induced Ca2+ release between mouse and human eggs

Storey Anna¹; Wang Yisu²; Elgmati Khalil²; Knaggs Paul¹; Swann Karl²

¹Wales Fertility Institute; ²Cardiff University

Background: At fertilization in mouse and human eggs activation is caused by repetitive Ca²⁺ oscillations which are initiated by phospholipase-Czeta that generates inositol-1-4-5-trisphosphate (InsP3). Previous work suggests that mouse eggs are more sensitive to phospholipase-Czeta than human eggs [1]. We have investigated this difference in sensitivity by stimulating eggs with Sr²⁺ or InsP3.

Methods: Ca²⁺ was measured in mouse eggs, or human eggs that had failed to fertilize after IVF or ICSI using dextran linked fluorescent dyes[2]. InsP3 receptor (IP3R) sensitivity was tested by uncaging InsP3 [3], and ATP measured using luciferase[2].

Results: Mouse eggs incubated in Ca²⁺-free, Sr²⁺-containing medium (5mM) immediately underwent Ca²⁺ oscillations (19/23). Human eggs failed to undergo any Ca²⁺ oscillations in Sr²⁺ medium (10mM) for >4 hours (n=21). In normal Ca²⁺-containing medium the sustained microinjection of Sr²⁺ also triggered Ca²⁺ oscillations in all mouse eggs (n=15) but not in human eggs (n=5). Since Sr²⁺ stimulates the IP3R[3], these data suggest the IP3R is less sensitive in human eggs. We tested IP3R sensitivity directly by photo-release of caged InsP3. Half maximal Ca²⁺ release was triggered by UV uncaging pulses of duration 0.26±0.031 seconds (n=13) in mouse eggs, compared with 6.9±0.18 seconds (n=14) in human eggs. Mouse and human eggs had similar resting Ca²⁺ levels. However, the concentration of ATP was consistently higher in mouse eggs compared to human eggs (3.32±0.07mM, n=37, vs 1.43±0.06mM, n=38). When ATP levels were lowered in mouse eggs by incubation in carbohydrate free medium, 5mM Sr²⁺ failed to cause Ca²⁺ oscillations. When pyruvate was added back to mouse eggs in this medium, ATP levels increased and Ca²⁺ oscillations were induced.

Conclusions: These data suggest that the IP3R is more sensitive to Sr^{2+} and InsP3 in mouse eggs compared to human eggs. This may be due to differences in the cytosolic concentration of ATP.

1.Swann K. (2018) The role of Ca^{2+} in oocyte activation during in vitro fertilization: insights into potential therapies for rescuing failed fertilization. BBA Cell & Mol. Res. 1865: 1830-1827 2.Campbell, K. and Swann, K. (2006) Ca^{2+} oscillations stimulate an ATP increase during fertilization of mouse eggs. Dev. Biol. 298: 225-233 3.Sanders J, Ashley B, Moon A, Woolley TE and Swann K. (2018) PLC γ induced Ca^{2+} oscillations in mouse eggs involve a positive feedback cycle of Ca^{2+} induced InsP3 formation from cytoplasmic PIP2. Front. Cell & Dev. Biol. 6: 36.

P182 Age and stage specific sensitivity of prepubertal mouse oocytes to vitrification-warming

Daddangadi Prabhakara Akshatha¹; Uppangala Shubhashree¹; Kalthur Guruprasad¹; Talevi Riccardo²; Adiga Satish Kumar¹

¹Department of Clinical Embryology, Kasturba Medical College Manipal, Manipal Academy of Higher Education Manipal; ²Dipartimento di Biologia, Università di Napoli "Federico II", Complesso Universitario di Monte S Angelo, Napoli, Italy

Purpose: To understand the age and maturation stage specific sensitivity of prepubertal mouse oocytes to vitrification

Methods: To mimic prepubertal conditions in an experimental setup, healthy female siblings of Swiss albino mice were sacrificed on postnatal days 14, 21 and 28 and their oocytes collected. The germinal vesicle (GV) stage oocytes and in vitro matured MII oocytes were subjected to vitrification-warming procedure. Both structural (meiotic spindle morphology, mitochondrial integrity, cortical granules) and functional (sperm zona binding, fertilization) characteristics were assessed in oocytes post vitrification-warming

Results: The process of IVM was found to be more detrimental to prepubertal oocytes than to young adults. Further, subjecting the prepubertal IVM oocytes to vitrification-warming resulted in an increase in the number of abnormal meiotic spindles, a change in the cortical distribution pattern, a reduction in sperm zona binding and fertilization rate. Importantly, IVM was found to be more successful after vitrification-warming of prepubertal GV oocytes than before

Conclusion: Results from this study are in favor of GV stage vitrification for prepubertal oocytes requiring fertility preservation. Understanding the mechanisms behind poor outcomes in IVM oocyte vitrification will help in refining the current protocol, thereby retaining the oocyte's maximum structural and functional integrity

P183 Distinct modes of chromosome lagging in meiosis-I predict aneuploidy in mouse oocytes

Mihajlovic Aleksandar; Mehrotra Caitlin; Fitzharris Greg

CRCHUM, Université de Montréal, Montréal, Canada

Background: The incidence of chromosome segregation errors that cause aneuploidy in the first meiotic division (MI) increases with age and is considered a major contributing factor of age-related decline in female fertility. A frequently observed segregation defect in aged oocytes is lagging anaphase chromosomes, though the extent to which these directly contribute to aneuploidy in mouse oocytes is unclear.

Methods: To understand the importance of lagging chromosomes in generation of aneuploidy, we combined live and fixed confocal fluorescence microscopy to monitor chromosomal behavior and examine their attachments to spindle-microtubules during MI.

Results: Live imaging revealed an increased incidence of lagging chromosomes during anaphase in old versus young oocytes (58.8% vs. 26.7%). Surprisingly, not all lagging chromosomes had common origin and exhibited identical behavior. Rather, we identified two distinct types of lagging chromosomes that we refer to as 'canonical', that originated from aligned bivalents, and 'non-canonical' that originated from mildly misaligned bi-oriented bivalents. Importantly, the presence of 'canonical' lagging chromosomes, reminiscent of those found in mitotic cells, strongly correlated with aneuploidy outcome of anaphase, while the presence of 'non-canonical' ones showed no correlation. Interestingly, the examination of chromosome attachment status shortly before anaphase revealed that in both young and old oocytes, the proportion of incorrect/merotelic attachments, considered responsible for producing lagging

chromosomes, was negligible. However, the proportion of chromosomes lacking stable microtubule attachments remained constantly high (20-30%) throughout entire MI in old oocytes. At the onset of anaphase, all chromosomes in both groups become stably attached to microtubules, suggesting that a large proportion of chromosomes in old oocytes rapidly stabilize their attachments just prior to anaphase.

Conclusion: We propose that this sudden formation of stable microtubule attachment might be highly error-prone thus giving rise to 'canonical' lagging chromosomes that would be responsible for aneuploidy in old oocytes.

P184 A mouse embryonic stem cell model reveals maternal protein restriction around conception alters embryonic signaling and metabolic phenotype

Khurana Pooja¹; Cox Andy²; Islam Barira³; McHugh Patrick³; Smyth Neil¹; Fleming Tom¹

¹University of Southampton; ²University of Cambridge; ³University of Huddersfield

Background: Maternal malnutrition around conception increases risk of offspring cardiometabolic disease¹. Our mouse model of maternal low protein diet in vivo over 0.5- 3.5 days to blastocyst (Emb-LPD) alters embryo phenotype and programmes changes in growth and physiology through gestation culminating in postnatal disease². Here, we use mouse embryonic stem cell (mESC) lines from Emb-LPD and control blastocysts to investigate in vitro underlying mechanisms of the origin of adverse programming.

Materials and Methods: (i) mESC lines were derived from blastocysts from dams fed isocaloric Emb-LPD (9% casein) or normal diet (NPD, 18% casein); (ii) Male lines were characterized for derivation, karyotype, pluripotency, proliferation, apoptosis and cell-cycling activity; (iii) Karyotypically normal lines were analysed by metabolomics, enzymatic activity and transcriptomics.

Results: Emb-LPD mESC lines had reduced derivation efficiency (20% vs 49% NPD; $P < 0.01$). While pluripotency marker expression, cell proliferation and cycling were unchanged between treatments, Emb-LPD lines displayed increased dead/apoptotic cells ($P < 0.05$) accompanied by reduced pERK 1/2 ($P < 0.05$) survival signalling activity and differential expression ($P < 0.001$) of MAPK pathway genes (including Maff, Atf4, JunD, Dusp8) following RNA-seq. analysis. Global metabolomic profiling identified Emb-LPD alterations in glucose metabolism, fatty acid homeostasis and ascorbate utilization. Emb-LPD lines exhibited increased glucose 6-phosphate (G-6-P) and fructose 6-phosphate ($P < 0.05$), reduced downstream metabolites ($P < 0.1$ trend), and reduced glycolytic enzyme activity of phosphofructokinase (PFK, $P < 0.05$) and differential expression of Gpi (G-6-P isomerase, $P < 0.001$), Pk (pyruvate kinase, $P < 0.001$) and facilitated fructose transporter (GLUT5, $P = 0.06$) which collectively may explain 'log-jam' accumulation of upstream glycolytic metabolites.

Conclusion: mESC models show suitability for analysing periconceptional developmental programming mechanisms, reducing animal numbers required and overcoming limited material in preimplantation embryos. We demonstrate Emb-LPD induces increased apoptosis, perturbed MAPK and ERK 1/2 signaling, and dysfunctional metabolism especially in the glycolytic pathway. This insight will permit more directed screening of embryos for origins.

1. Fleming TP, Watkins AJ, Velazquez MA, Mathers JC, Prentice AM, Stephenson J, et al. Origins of lifetime health around the time of conception: causes and consequences. *The Lancet*. 2018.

2. Fleming TP, Watkins AJ, Sun C, Velazquez MA, Smyth NR, Eckert JJ. Do little embryos make big decisions? How maternal dietary protein restriction can permanently change an embryo's potential, affecting adult health. *Reprod Fertil Dev*. 2015;27(4):684-92.

P185 Proper structure of the oocyte-cumulus extracellular matrix depends on the interaction between hyaluronan and inter-alpha-trypsin inhibitor proteins in ovarian follicles

Nagyova Eva; Vodicka Petr

Institute of Animal Physiology and Genetics, Czech Academy of Sciences, Libeň, Czech Republic

Background: Extracellular matrix (ECM) is an important structure that is present in all tissues. The ECM interacts with cells to regulate a wide range of functions, including adhesion, proliferation, apoptosis and differentiation. The ECM can also locally release growth factors, such as epidermal growth factor, fibroblast growth factor, and other signaling

molecules such as transforming growth factor and amphiregulin. After gonadotropin stimulus, cumulus cells expand and form hyaluronan (HA)-rich cumulus ECM. In ovarian follicles, the proper structure of the cumulus ECM depends on the interaction between HA and serum-derived proteins of the inter-alpha- trypsin inhibitor family. Previously, we have evaluated the covalent linkage of heavy-chains of inter-alpha-trypsin inhibitor family proteins to HA, as the principal component of the expanded HA-rich cumulus ECM in porcine oocyte-cumulus complexes (OCC). Aim: The aim of this study was to investigate a spatiotemporal expression of inter-alpha-trypsin inhibitor family in porcine ovarian follicles.

Methods: Porcine OCC stimulated in vivo and in vitro with gonadotropins were analyzed by confocal and immunofluorescence microscopy. Proteins of the inter-alpha-trypsin inhibitor family were detected using rabbit anti-human inter-alpha-trypsin inhibitor antibody (DAKO, Carpinteria, CA).

Results: Both, in vivo and in vitro gonadotropin-stimulated OCC cultured in serum-supplemented medium accumulated inter-alpha trypsin inhibitor proteins in the expanded HA-rich cumulus ECM. In contrast, OCC cultured in medium without serum (cultured in polyvinylpyrrolidone-supplemented medium) were not able to form cumulus ECM.

Conclusion: Our results confirm that HA-rich oocyte-cumulus ECM does not form in serum-free conditions, while it does in the presence of serum-derived inter-alpha-trypsin inhibitor family proteins.

P186 Single strand DNA-binding proteins (Ssb1 and Ssb2): Novel determinants of oocyte development and female fertility

Subramanian Goutham Narayanan; Homer Hayden

University of Queensland - Centre for Clinical Research

Background: Bidirectional communication between oocytes and follicular cells is crucial for acquisition of oocyte developmental competence. Regulated transcription¹ during oocyte growth stockpiles polyadenylated mRNA and protein reserves for supporting follicle development and ultimately, embryogenesis, prior to embryonic genome activation. Very little is known about oocyte-specific master regulators that direct both transcription and late-stage follicle development. In somatic cells, Single strand DNA-binding proteins, Ssb1 and Ssb2, play overlapping roles in DNA repair² and regulating expression of polyadenylated mRNAs³. To-date, the roles of Ssb1/2 in oocyte development and female fertility remain unknown.

Methods: We generated oocyte-specific Ssb1/2 double-knockout (Ssb1/2^{-/-}) mice for the first time. We studied the effects on female fertility using mating trials; ovarian histology; RT-PCR, western blotting and timelapse imaging of in vitro oocyte maturation.

Results: We find that Ssb1/2^{-/-} females are infertile, producing no pups after 6 mating rounds compared with ~8-9 pups/litter from Ssb1/2^{flox/flox} females. There is a specific reduction in pre-ovulatory antral follicles in Ssb1/2^{-/-} ovaries with all other follicle stages remaining intact. Consistent with this, immature oocytes obtained from hormonally primed Ssb1/2^{-/-} mice are significantly smaller than those from control mice, pointing to a defect in final oocyte growth. Significantly, the oocyte-specific factors Bmp15, Gdf9, Fgf8 and c-Kit are down-regulated in Ssb1/2^{-/-} oocytes. Moreover, levels of cyclin-dependent kinase 1 (Cdk1) co-activator, cyclinB1 are also markedly reduced. Consistent with the essential role of Cdk1 activation for M-phase entry, we find that Ssb1/2^{-/-} oocytes remain chronically arrested at the G2-stage.

Conclusions: Here we find that oocyte Ssb1/2 is indispensable for female fertility. Our findings are consistent with Ssb1/2 being novel upstream regulators of key oocyte-specific factors required for late antral-stage follicle development and oocyte growth. Consequently, infertility following loss of Ssb1/2 is not due to depleted oocyte numbers, but to severe impairment of oocyte developmental competence.

1. Wassarman, P. M. & Kinloch, R. A. Gene expression during oogenesis in mice. *Mutation Research/Reviews in Genetic Toxicology* 296, 3-15, doi:[https://doi.org/10.1016/0165-1110\(92\)90028-8](https://doi.org/10.1016/0165-1110(92)90028-8) (1992). 2 Shi, W. et al. Ssb1 and Ssb2 cooperate to regulate mouse hematopoietic stem and progenitor cells by resolving replicative stress. *Blood* 129, 2479 (2017). 3 Skaar, J. R. et al. The Integrator complex controls the termination of transcription at diverse classes of gene targets. *Cell Research* 25, 288, doi:10.1038/cr.2015.19. <https://www.nature.com/articles/cr201519#supplementary-information> (2015).

P187 Classification of embryos at the morula stage is highly correlated with live birth outcome

Montgomery Sue¹; Campbell Alison²; Foad Fiona³; Davies Laina⁴; Best Louise²; Davis Natalie⁵; Smith Rachel²; Duffy Sam¹; Thirby-Moore Sarah⁶; Wheat Stacy⁷

¹CARE Fertility Manchester; ²CARE Fertility; ³CARE Fertility Tunbridge Wells; ⁴CARE Fertility Nottingham; ⁵CARE Fertility Northampton; ⁶CARE Fertility Birmingham; ⁷CARE Fertility London

Introduction: Evidence from conventional microscopy has previously suggested that if cellular material is excluded from the embryo at the compaction stage (incomplete compaction), blastocyst formation and therefore outcome may be compromised (Ivec et. al., 2011). The introduction of time-lapse technology has made it possible to evaluate the compaction process thoroughly, due to the ability with time-lapse to observe the entire process rather than rely on a single static observation. This has enabled any correlation between compaction completeness and morula grade and live birth outcome to be studied.

Materials and Methods: Using the time lapse incubator, Embryoscope (Vitrolife) 2,612 blastocysts of known live birth outcome (KID) from 7 sister clinics were scored according to the percentage of excluded material identified at the point where compaction was at its maximum, just prior to morula formation. The embryos were classified as either M1 (complete compaction with all cellular material included) or M2 (some cellular material excluded). This classification at the morula stage was then compared to live birth outcome using the Fishers Exact test.

Results: Of the 2,612 embryos considered 232 were excluded as the compaction grade had not been scored at the time of morula formation. Of the remaining 2,380 embryos 1,109 (47%) were classified as M1 and 1,271 (53%) were classified as M2. Those embryos that exhibited complete compaction (M1) had a significantly higher live birth rate of 38% (421/1109) compared to those that were graded as M2 at 21% (266/1271) ($p=0.0001$). Further data comparing morphokinetic model grade with compaction grading will also be presented.

Conclusion: Full evaluation of the compaction process which can only be achieved using time lapse can significantly improve the selection of the embryo from a cohort that is most likely to result in a live birth.

P188 Types of vacuolation, in the human preimplantation embryo and association with live birth

Smith Rachel¹; Campbell Alison¹; Montgomery Sue²; Best Louise¹; Foad Fiona³; Davis Natalie⁴; Duffy Samantha²; O'Boyle Sarah⁵; Thirlby-Moore Sarah⁶; Wheat Stacy⁷; Davies Laina⁸

¹CARE Fertility; ²CARE Manchester; ³CARE Tunbridge Wells; ⁴CARE Northampton; ⁵Beacon CARE Fertility; ⁶CARE Birmingham; ⁷CARE London; ⁸CARE Nottingham

The presence of vacuoles at different stages in the developing embryo has been reported to affect blastulation rates (1). Time-lapse imaging has enabled closer monitoring of this phenomenon. Vacuoles can present singularly or as multiple membrane bound fluid inclusions. The incidence and possible impact of single and multiple vacuoles has been assessed, relative to live birth data. Using Time-lapse imaging, Embryoscope (Vitrolife, Sweden), 4462 transferred blastocysts with known live birth outcome (KID) were analysed from 7 sister clinics to assess the impact of single or multiple vacuoles on live birth outcome, using logistic regression. The presence of vacuole type was identified from time-lapse images up to the time of blastocyst transfer. The time of the appearance of the vacuoles was categorised as late >72 hours post insemination (hpi) and early ≤72hpi. From 4462 transferred blastocysts, the incidence of single vacuoles was 2.6% (n=119) and multiple vacuoles 5.3%, (n=239). The live birth rate (LBR) for transferred blastocysts where multiple vacuoles were present was significantly lower than when no vacuoles were recorded, 20.9% vs 31.5% ($p=0.01$). Single vacuolated transferred blastocysts LBR was 28.5% vs 31.5% (NS). The appearance of both vacuole types in the late stage (>72hpi) resulted in significantly lower LBR compared to the rest of the cohort 12.3% vs 31.5% ($p=0.01$). Early appearance of vacuolation was not shown to be associated with reduced viability, LBRs were 27.1% vs 31.5%. Blastocysts exhibiting multiple vacuoles were associated with significantly lower live birth outcome compared with embryos not exhibiting vacuolation. The presence of single vacuoles was not significant in this analysis. The time the vacuoles appeared was critical, single and multiple vacuoles appearing in the later stage (>72hpi) were associated with significantly reduced live birth outcome when compared with earlier appearance.

1. Ebner T, Moser M, Sommerguber M, et al. Occurrence and developmental consequences of vacuoles throughout preimplantation development. *Fert Steril*. 2005;83:1635-1640.

P189 Can blastocyst percentage of re-expansion after warming be used to predict implantation potential and live birth outcome?

Miralles Cuadrado Carmen¹; Mirzazadeh Fatima¹; Khairy Mohammed¹; Armstrong Ellen²; Lockwood Megan³; Nice Lynne¹; Campbell Alison⁴; Best Louise⁴

¹CARE Fertility Northampton; ²CARE Fertility Birmingham; ³CARE Fertility Sheffield; ⁴CARE Fertility

Introduction: The aim of this study was to corroborate if there is a correlation between blastocyst re-expansion after warming, and implantation and live birth. We aimed to develop a model to predict the implantation and live birth potential of post warmed blastocysts.

Materials and methods: We retrospectively studied the re-expansion rates for 703 blastocysts, blind to outcome. All embryos included had known implantation data (KID), the number of transferred embryos matched the number of foetal hearts. 242 were KID positive vs 461 KID negative. Of these 164 resulted in LB vs. 483 LB negative. Blastocysts were warmed using vitrification media (Kitazato, Japan) and placed into a time-lapse incubator. Blastocyst diameter was measured post warming at times 0 and 90 minutes. The percentage of re-expansion was calculated for each blastocyst at time interval 0-90 minutes. At 90min post warming, blastocysts with a positive percentage of re-expansion were grouped as "re-expanded", and blastocyst with 0 or negative percentage of re-expansion were grouped as "collapsed".

Results: Mean blastocyst diameter at 90 minutes and mean percentage of re-expansion 0-90 minutes showed a statistical significant correlation for implantation and LB. The group 're-expanded' presented a higher implantation and LB, but was not statistically significant. A logistic regression analysis for LB prediction has shown significant effect of age of the patient, mean blastocyst diameter and percentage of re-expansion. A post regression estimation analysis showed that a minimum of 20% re-expansion is required for optimum LB above 25%, progressively increasing the greater the re-expansion.

Conclusion: This study demonstrated that blastocyst diameter measurements at 90 minutes post warming and the percentage of re-expansion are significantly correlated with implantation and LB outcomes. The use of a predictive model that correlates the blastocyst percentage of re-expansion post warming and LB could be used as a tool to make clinical decisions.

P190 A prospective randomized sibling-oocyte study of two uninterrupted media systems for culturing blastocysts: impact on blastulation, implantation and ongoing pregnancy rates

Calonge Marcela¹; Marin Diego²; Machuca Dafne¹; Morales Blanca¹; Meseguer Marcos³; Troncoso Carlos¹

¹IVI Santiago de Chile; ²Basking Ridge, New Jersey; ³IVI Valencia

Objective: Currently, commercial media are available in different formulations to support specific stages of embryo development. Culture of embryos is possible under different conditions: whether the media is refreshed, changed or left undisturbed for the 5-6 days of embryo culture. Some studies have shown that uninterrupted culture is effective and brings acceptable results. In addition, uninterrupted culture facilitates the workflow in the laboratory by eliminating several steps of moving embryos. Given the variety of available monophasic media formulations, this study aimed to evaluate the performance of two different formulations of uninterrupted culture media in terms of blastulation rates and clinical outcomes.

Design: Prospective randomized study.

Materials and Methods: Patients undergoing IVF between November 2017 and December 2018 in a single center with at least 7 mature (MII) oocytes after retrieval were included in the study. Patients whose partner presented severe male factor were not included. All MII oocytes from each cycle's cohort were equally randomized before ICSI to be cultured under either one of the two uninterrupted media formulations tested: Vitrolife or Lifeglobal. Embryo transfers

were performed in D5 or D6. Primary endpoints were blastulation, implantation and ongoing pregnancy rates. Fisher's test and Poisson regression model using oocyte age as covariate were computed appropriately.

Results: 137 study subjects participated in the study, from which 108 underwent an oocyte donation cycle. . After computing the statistical analysis, all primary endpoints were comparable between the two culture media formulations in question (Table 1).

Conclusion: Our data indicates that uninterrupted embryo culture is a feasible strategy with satisfactory outcomes. This is encouraging since uninterrupted culture minimizes embryo manipulation and improves the IVF lab workflow, which is essential to boost quality in IVF laboratories.

1. Biggers, John D. et al. Choosing a culture medium: making informed choices. *Fertility and Sterility*, 2008. Volume 90, Issue 3, 473 - 483 2. Morbeck DE, Krisher RL, Herrick JR, Baumann NA, Matern D, Moyer T. Composition of commercial media used for human embryo culture. *Fertil Steril*. 2014 Sep;102(3):759-766.e9. Epub 2014 Jul 4. 3. Reed ML1, Hamic A, Thompson DJ, Caperton CL. Continuous uninterrupted single medium culture without medium renewal versus sequential media culture: a sibling embryo study. *Fertil Steril* 2009;92:1783-6. 4. Schneider D, Verza S, Esteves S. A single medium and sequential media are equally effective for the culture of human embryos to the blastocyst stage: a pilot study. *Fertil Steril*. 2009, Volume 92, Issue 3, Supplement, Pages S231-S232 5. R. Singh, E. Johnston-MacAnanny, T. Yalcinkaya, A. J. Carrillo. Ob/Gyn, Wake Forest University Baptist Medical Center, Winston-Salem, NC. BLASTOCYST (blast) DEVELOPMENT OF SIBLING EMBRYOS IN A SINGLE CONTINUOUS EXTENDED CULTURE (CEC) VS. SINGLE CULTURE MEDIA RENEWED ON DAY3 (CMR). *Fertil Steril*, 2012, Volume 98, Issue 3, Supplement, Page S106 6. N. Desai1, Goldberg Jeffrey1, Austin Cynthia1, Falcone Tommaso1. Global single step medium for uninterrupted culture to blastocyst in the EmbryoScope: examination of clinical outcome and live birth data from blastocyst transfer cycles 7. L. Nolte, N. Silver1, T. Lundberg1, F. Hambiliki1, M. Bungum1. P-509 Embryo culture in a continuous single medium and selection by morphokinetic analysis: a prospective randomized sibling oocyte trial of the embryo scope and primo vision.

P191 Blastocyst cell membrane lipid profile can be changed upon the co-culture with multilamellar vesicles: a new strategy to modulate lipids in embryos?

Zoccal Mingoti Gisele¹; De Rossi Hugo¹; Teixeira Rodrigues Rossi Luana¹; Barros Nunes Giovana¹; Lemos Monique¹; da Silva Feitosa Eloi¹; Fernanda de Oliveira Rocha Daniele²; Colnaghi Simionato Ana Valeria²; Benites Aoki Pedro Henrique¹; Gouveia Nogueira Marcelo F¹

¹São Paulo State University (UNESP); ²The University of Campinas (UNICAMP)

In vitro produced (IVP) embryos show reduced cryotolerance and alteration of cell membrane lipid profile when compared to their in vivo counterparts. Cell membrane unsaturated lipids are essential to confer high fluidity during the cryopreservation process. Multilamellar vesicles (MLVs) are self-assembled lipid bilayers that have successfully mimetized cell membranes in different applications, including as carriers to a target-cell. Herein we shall evaluate the co-culture of MLVs with IVP bovine embryos aiming to alter the lipid profile of the embryonic cell's membrane. The MLVs were produced with phosphatidylcholines and probed under different concentrations (1.0, 1.5 and 2.0 mmol/L) in ultrapure water and culture medium regarding their stability in the culture conditions (Dynamic Light Scattering) and embryotoxicity. The co-culture of MLVs with bovine embryos (from Day 5 to Day 7 post-insemination) was used as a model for modifications on the cell membrane lipid profile of Day 7 blastocysts (MALDI-TOF/MS). Independently of the concentration, the MLVs were stable for 48 hours on culture conditions although those synthesised with water had a pronounced decrease in average diameter (from 946.91 to 654.80 nm respectively for 0 and 48 hours). There was no statistically observed toxic effect on the embryo production even with the highest concentration (47.3 and 38.1% of blastocyst, respectively for control and 2.0 mmol/L). Those concentration of MLVs induced a distinct lipid profile in the blastocyst cell membrane compared to the control, mainly on phosphatidylcholine (PCe 32:0; PC 32:1 or PC 34:4; PC 34:2; and PC 34:2 or 36:5), and glycerophospholipids (PG 42:4, and PG 44:5) ions. The use of MLVs during the last 48 hours of in vitro culture was able to induce direct and/or indirect changes of lipid profile of embryonic cell membrane. Further evaluation on post-vitrification survival and pregnancy establishment is ongoing. Grant #2012/50533-2, São Paulo Research Foundation (FAPESP).

P192 Using morphokinetics to determine which high quality blastocyst to select for transfer

Florek Agnieszka; Pool Georgina; Rogers Shaun

GENNET City Fertility

Objective: Assess morphokinetic data to identify whether particular parameters aid the selection of the most-likely-to-implant embryo, out of a pool of morphologically similar blastocysts.

Methods: A retrospective analysis of morphokinetic parameters was performed on embryos of known outcome for implantation (KID) to identify whether different patterns can be seen. The blastocysts selected for study were transferred in both fresh and frozen cycles between February 2013 - September 2019. Time-lapse morphokinetic analysis was performed on 222 embryos. A traditional, but comprehensive grading system was also employed (a modified Istanbul consensus grading system was used for cleavage stage embryos and the Gardner grading was utilised for blastocysts [1,2]). High quality KID positive blastocysts (n = 112) with grade A or B inner cell mass and trophectoderm were analysed and the data compared against values seen for comparable KID negative embryos (n = 110).

Results: In agreement with established literature [2,3], KID positive embryos had better inner cell mass, trophectoderm, day 2, and day 3 grades than KID negative embryos ($P < 0.05$ in all cases).

KID positive embryos start blastulation (tSB), reach full blastocyst stage (tB) and become expanded (tEB) significantly sooner than KID negative embryos ($P < 0.05$).

However, for the blastocysts with high day 3 grades, only tEB proved to be a statistically significant indicator for higher implantation potential ($P < 0.05$).

Conclusions: The morphokinetic parameters tSB, tB and tEB can help guide embryo selection among otherwise similarly graded high quality blastocysts. This approach can allow the embryologist to make a more accurate decision about the selection of a blastocyst with the greatest implantation potential, in conjunction with traditional grading systems.

[1] Alpha Scientists in Reproductive Medicine and ESHRE Special Interest Group of Embryology. The Istanbul consensus workshop on embryo assessment: proceedings of an expert meeting, Hum Reprod, 2011, vol. 26 (pg. 1270-1283).

[2] Gardner DK, Lane M, Stevens J, Schlenker T, Schoolcraft WB. Blastocyst score affects implantation and pregnancy outcome: towards a single blastocyst transfer. Fertility and sterility. 2000 Jun 1;73(6):1155-8.

[3] Meseguer M, Herrero J, Tejera A, Hilligsøe KM, Ramsing NB, Remohí J. The use of morphokinetics as a predictor of embryo implantation. Human reproduction. 2011 Aug 9;26(10):2658-71.

P193 Is the increase in success rate overtime observed in the HFEA register associated with increased proportion of blastocyst transfers?

Hickman Cristina; Jacques Celine; Barkaoui Samya; Louzada Julio; Noublanche Caroline; Beaumont Lucy; Kotrotsou Mara

Apricity

Introduction: IVF clinics worldwide have increased the proportion of cycles undergoing blastocyst culture. Blastocyst culture in particular has been recently implicated as one of the causes in worldwide decline of IVF birth rates (Gleicher et al. 2019). The objective of this study was to identify whether increase in success rates over time observed in the HFEA register could be explained by the increase in blastocyst transfer.

Methods: Population based cohort study using the HFEA register (N=316,029 cycles), from 2000 to 2016. For this period, Day 1,2,3,4,5,6 ET occurred in 0.4,42,32,1,24,1% of cycles, respectively.

Results: From 2000 to 2016, live birth per cycle started (LBR) across all age groups increased from 24% to 31.5%, ($p < 0.001$) and the median day of embryo transfer increased from 2.3 to 4.1 ($p < 0.001$). From 2000 to 2005, LBR for blastocyst culture increased from 25% to 50% ($p < 0.001$). From 2005 to 2016, the LBR for blastocyst ET remained stable. Day 2 and 3 ET LBR remained stable from 2000 to 2008, 25%, with a 10% reduction in LBR from 2008 to 2016. Delaying embryo transfer increased LBR in a similar manner from day 1 to day 5 across all age groups (18-34, 35-37, 38-39, 40-42, 43-44, NS). Within the group of patients where only 1 embryo was created, the trend of increase in success rate with increasing day of transfer was also observed across the age groups ($p < 0.001$).

Conclusion: According to the HFEA register, based on LBR only, irrespective of patient age and number of embryos created, blastocyst transfer should be offered instead of cleavage transfer to maximise LBR. Neo-Natal and post-natal health outcomes were not the focus of this study, and should also be considered when determining culture strategies.

P194 CPEB2 mediates stabilization and subcellular localisation of TJP1 mRNA during mouse blastocyst formation

Choi Inchul; Lee Seulah

Chungnam National University

Cytoplasmic polyadenylation element binding protein 2 (CPEB2) is known to disrupt tight-junction (TJ) assembly via mislocalisation of tight junction protein 1(TJP1) and depletion of CPEB2 results in retarded blastocyst formation in pig embryos (1, 2). However, the mechanism of CPEB2 mediated regulation is not examined during preimplantation development. The aim of this study was to elucidate whether CPEB2 affect poly(A) tail stabilization and trans-localisation of TJP1 mRNA during morula and blastocyst transition. Here, we showed that CPEB were detected in nuclei at the early cleaving stages and then localised at the apical cell membrane from morula onwards. RNA fluorescence in situ hybridization demonstrated that TJP1 mRNAs were localised apically in the control morula, but not in the CPEB knockdown embryos. We also examined poly(a) tailing length using bioanalyzer electrophoresis. The length of poly(a) tailing of Glyceraldehyde 3-phosphate dehydrogenase(G3pdh increased in the control and the KD embryos after poly(a) tailing and the fragments were not different between them. However, the length in the KD embryos were relatively shorter (about 281 bp) compared to the control embryos (from 281 bp to 317 bp). These results indicated that CPEB2 may directly regulate efficiency of translation and localization of TJP1 and affect tight junction assembly during mouse blastocyst formation

1. Nagaoka K, Udagawa T, Richter JD. CPEB-mediated ZO-1 mRNA localization is required for epithelial tight-junction assembly and cell polarity. Nat Commun.2012 Feb 14;3:675. doi: 10.1038/ncomms1678. 2. Kwon J, Park S, Seong MJ, Choi I, Kim NH. Corrigendum to: Cytoplasmic polyadenylation element binding protein 2 (CPEB2) is required for tight-junction assembly for establishment of porcine trophectoderm epithelium. Reprod Fertil Dev. 2019 Mar;31(3):632. doi: 10.1071/RD18098_CO.

P195 Association between blastocyst grade and day of biopsy and euploidy rates

Stavarakis Thomas; McCollin Abeo; Al-hashimi Balsam; Gorodeckaja Julija; Ahuja Kamal; Macklon Nick; Summers Michael

London Women's Clinic

Purpose: To determine the relation between blastocyst grade, day of biopsy and euploidy rate in women of different age groups. Design: Retrospective analysis of trophectoderm (TE) biopsy for PGT-A using Next Generation Sequencing (NGS). Four embryo grades: excellent(E), good(G), fair(F) and poor(P).

Materials and Methods: 182 women, 29-45 years (mean 38.7±3.4) underwent IVF PGT-A. TE biopsy of 993 blastocysts were analysed. Logistic Regression Analysis was used to assess blastocyst grading and day of biopsy on the euploidy rate.

Results: Proportion of embryos biopsied: 56% on day 5, 36% on day 6 and 8% on day 7. Euploidy rates were 37%, 20% and 13% of day 5, 6 and 7 biopsied embryos, respectively. Euploidy rates were 63%, 49%, 35% and 19% of embryo morphology scores, E, G, F and P, respectively. For women < 35y, 35-37y, 38-39y, 40-41y and 42-45y with P embryos the euploid rate was 38%, 28%, 26%, 13% and 6%, respectively. Euploidy rates, same age groups, for G and F were 61%, 58%, 47%, 33% and 17%, respectively. Across all age groups, the day of biopsy and embryo grading score were significantly negatively correlated with the chance of euploidy ($r=-0.2$ and -0.255 , respectively, $p<0.01$) The age of the patient has no statistically significant impact on the biopsy day, whereas age and the day of the biopsy have statistically significant impact on the euploidy.

Conclusions: Morphological grading of blastocysts is positively correlated with the euploidy rate and explains the lower implantation rate seen with poor quality embryos. Morphologic grading of embryos alone cannot select euploid embryos for transfer. Moreover, poor quality embryos can be euploid and achieve similar implantation and pregnancy rates as good quality euploid blastocysts. In the setting of no PGT-A one might want to take the view that 'every embryo deserves a chance'.

P196 Progesterone levels on the day of euploid frozen embryo transfer following preimplantation genetic testing do not impact livebirth rate

Theodorou Efsthios; Ben Nagi Jara; Ozturk Ozkan; Heath Carleen; Cawood Suzanne; Serhal Paul

The Centre for Reproductive & Genetic Health

Purpose: To evaluate the impact of serum progesterone (P4) levels the day of euploid frozen blastocyst transfer on ongoing pregnancy/live birth rate (OP/LBR).

Methods: A retrospective analysis was carried out on frozen-thawed embryo transfers (FTET) of euploid blastocyst(s) in a single centre from 2015 to 2017. All embryos were biopsied and vitrified at blastocyst stage for preimplantation genetic testing for monogenic (PGT-M) or structural chromosomal rearrangement (PGT-SR) and/or for aneuploidy (PGT-A). PGT-A was also performed on all embryos subjected to PGT-M/SR. Each patient's first medicated FTET cycle was included (N=279) where 1 or 2 euploid blastocysts were transferred, supplemented according to unit protocol with injectable hydroxy-progesterone, vaginal and oral progesterone. When P4 measured on embryo transfer day was below 100pmol/L, additional progesterone was started as per protocol.

Results: Mean P4 levels for cycles with an on-going pregnancy/live birth were similar to those cycles that did not (189.1±19.2 vs. 220.9±20.1 pmol/L, p=0.268).

Two groups based on P4 levels were compared (group A, P4 <100pmol/L; group B, P4>100pmol/L). OP/LBR was not significantly different between the two groups (59% vs. 56%, p=0.308). OP/LBR was not significantly different when P4 was further stratified into four groups based on P4 levels (Groups: <50, 50-100, 100-150, >150, OP/LBR was 50%, 62%, 54%, and 56% respectively).

Following multivariate analysis, the number of embryos transferred (OR 4.56, CI 1.61--12.94, p=0.004) and whether the embryos had PGT-M/SR vs PGT-A (OR 1.97, CI 95% 1.19-3.26, p=0.008) were significant factors. The downregulation protocol (with agonist or antagonist), age, the morphologic quality of the embryos transferred and the P4 levels (grouped in 2 or 4 categories) had no effect on OP/LBR.

Conclusions: P4 on the day of frozen-thawed euploid blastocyst(s) transfer after PGT-M/SR/A does not predict the chance of ongoing pregnancy or live birth. However, frozen embryo transfer cycles following PGT-M/SR/A have a higher OP/LBR than after PGT-A.

P197 How many oocytes are required to generate at least one euploid embryo?

Stavakis Thomas; Summers Michael; Gorodeckaja Julija; Al-hashimi Balsam; Linara-Demakakou Elena; Wang Jinjun; Ahuja Kamal; Macklon Nick

London Women's Clinic

Purpose: To determine the relationship between oocyte number and the chance of obtaining one euploid embryo in women of different age groups undergoing IVF treatment.

Design: A retrospective analysis of 488 patients undergoing IVF/PGT-A. 1669 blastocyst trophectoderm (TE) biopsies were examined using Next Generation Sequencing (NGS) between 2016 and 2019. Materials and Methods: 488 women between 28-45 years (mean 39.3±3.4) undergoing IVF and PGT-A. Logistic Regression Analysis was performed to assess the impact number of collected oocytes, age and AMH on the chances of having at least one euploid embryo.

Results: Overall, 27.5% of the biopsied blastocysts were euploid ranging from 45% in those under 35y to 9% in those 42-45y. 48% of the patients had at least one euploid embryo. In women under 35, obtaining less than 10 oocytes was associated with a 47% chance of having at least one euploid embryo, rising to 98% in those with 11-15 oocytes. In women aged 35-39, the respective figures were 52% and 79% and in women aged 40-41 the respective figures were

29%, and 47% respectively. In those 42-45y, only 17% with fewer than 10 eggs obtained a euploid embryo, rising to 55% in the rare cases when >20 eggs were obtained. AMH was a weaker predictor, but a value of > 20pmol/l was associated with 98%, 90%, 75%, 50% and 30% chance of having at least one euploid embryo for female age groups 28-34, 35-37, 38-39, 40-41, 42-45 years old respectively.

Conclusions: These data can support counselling on their likely chance of achieving at least one euploid embryo from a single cycle of IVF. Obtaining <10 eggs in women under 35 was associated with a lower chance of a euploid embryo than those 35-37, indicating that in young women low response may also indicate lower egg quality.

P198 Assessing the impact of maternal age on frozen embryo replacement outcomes

Manukian Barbara; Sarodey Geet; Saravelos Sotirios; Rattos Annabel; Jansa Marta; Salim Rehan

Wolfson Fertility Centre, Hammersmith Hospital, Imperial College NHS Trust

Background: Embryo vitrification allows cryopreservation with a high survival rate and comparable outcomes to fresh embryos. We aim to assess the impact of advancing maternal age on reproductive outcomes with cryopreserved embryos.

Methods: A retrospective analysis of consecutive patients who underwent frozen embryo transfer replacement cycle (FERC) between January 2013 and January 2019 at a single centre. Variables analysed included: Average age of eggs, pregnancy rate (PR), clinical pregnancy rate (CPR), live birth rate (LBR) and average birth weight (BW).

Results: 1,988 FERC cycles were analysed, 216 (10.8%) natural FERC and 1,772 (89.1%) medicated cycles. Embryos were frozen between 0 to 12 years. Patients were divided into four groups: embryos frozen for <1 (A), between 2-3 years (B), 4-5 years (C) and 6 or more years (D). There is no difference in average of age of egg between groups (average 32.7, range 32.4-33.6 years). Most patients had a FERC within one year (1,449), 356 in 2-3 years, 139 in 4-5 years and 44 after 6 years. There was no difference in PR (56.17% and 58.42%, $P>0.05$) and CPR (41.68% and 42.97%, $p>0.05$) between Group A and B. However PR (46.04% and 36.36%, $P<0.05$) and CPR (36.39% and 22.72%, $P<0.05$) were significantly lower in Group C and D compared to A and B. With respect to LBR, there was no difference in LBR in pregnancies within 5 years: 32.57% in those <1 year, 34.26% in 2-3 years and 30.21% in 4-5 years. Finally, in terms of BW, the average was similar in all groups (average 3,230 kg: range 3,151 -- 3,390 kg)

Conclusions: Advancing maternal age is associated with reduction in early reproductive outcomes (PR, CPR) but not late (BW, LB), after 3 years of storage. Our data is confounded by changes in laboratory protocols for vitrification which need to be considered in any conclusions.

P199 Does EmbryoGlue® have an impact on outcomes for fresh and frozen day 5 embryo transfers?

Fadon Patricia; Hill Kimberley; Abdo Dima; Breen Danielle

Thames Valley Fertility

Background: It has been reported that adherence compounds added to the embryo transfer medium such as those present in EmbryoGlue® increase the likelihood of embryo implantation, with the potential for higher clinical pregnancy and live birth rates⁽¹⁾.

Aim: to evaluate the effect of EmbryoGlue® on clinical pregnancy rates (CPR) and live births (LB) when compared with 1-step culture media in fresh and frozen embryo transfers (ET).

Methods: This retrospective study included 656 embryo transfers on day 5 with known CPR and LB. Data was divided into fresh or frozen ET using EmbryoGlue® or SAGE 1-step™ culture media, for both IVF and ICSI cycles. Statistical significance was calculated using the Chi-squared exact and Students t-test.

Results: The CPR for ETs (including fresh and frozen) using EmbryoGlue® was slightly higher when compared to 1-step media (28.0% vs 27.3% respectively) though not statistically significant. When comparing fresh and frozen ETs, CPR was slightly higher in the EmbryoGlue® group with the difference being higher in embryos originating from IVF cycles (31.7% vs 28.6%, respectively). LB was also slightly higher in frozen IVF ETs where EmbryoGlue® was used compared to SAGE 1-step™ (18.3% Vs 10.2% respectively) but not statistically significant.

Conclusion: Our data shows that the use of EmbryoGlue® may have a slightly positive impact on CPR and LB in frozen embryo transfers, especially when embryos were created by IVF however our data analysis showed no significance. A larger sample size is needed and other factors such as age and history should be taken into account to further validate the impact on CPR and LB.

⁽¹⁾Bontekoe, S, N Johnson, and D Blake. 2015. Adherence Compounds in Embryo Transfer Media for Assisted Reproductive Technologies (Review). Cochrane Database of Systematic Reviews, no. 2. <https://doi.org/10.1002/14651858.CD007421.pub3>. www.cochranelibrary.com.

P200 Choice of oil impacts the degree of osmolality change of single step IVF culture medium within non-humidified incubators

Gadd Stephanie

CARE Fertility Bath

Objective: To assess whether different types of oil can affect osmolality of culture medium droplets in non-humidified incubators. Despite an oil overlay, osmolality of medium is known to gradually increase if the culture environment is not humidified (1). Culture up to 6 days could lead to increases beyond the optimal physiological range and be detrimental to embryo development.

Methods: The performances of 3 commercially available oils for IVF were assessed. For each, 10 x 60mm culture dishes were prepared using 10 x 20µl droplets of culture medium overlaid with 8ml of oil, mimicking standard practice. Each individual dish was weighed immediately after preparation and again after 6 days' incubation in a non-humidified incubator at 37C. Final inferred osmolalities were calculated by measuring weight change due to water loss. The initial weight of 10 x 20µl droplets of medium is 0.2g per dish, equivalent to 0.054mOsm of solute at osmolality of 270mOsm/kg. Resultant osmolality was calculated by dividing 0.054 by the final weight (in kg) of the 10 droplets.

Results: The study showed a significant change in droplet weight of -12% for Oil A, resulting in an inferred culture medium osmolality of >300mOsm/kg after 6 days, outside the optimal working range. Although droplet weights under the other two oils also changed (by -8% for Oil B and by -6% for Oil C), the resultant inferred osmolality remained within the physiological range and limits.

Conclusions: Where IVF culture droplet dishes are used in non-humidified incubators it is important to consider the type of oil used in order to ensure optimal culture conditions. This may be even more critical where environmental humidity in the laboratory is also low, or air flow is high.

1. Media osmolality changes over 7 days following culture in a non-humidified benchtop incubator Swain JE et al. (2016) Fertil Steril 106 (3), e362

P201 Avoiding day 3 morphological assessment improves embryo utilisation rates with the use of a single step culture medium in standard culture incubators

Owen Sarah; French Emily; Mascarenhas Mariano; Thompson Karen; Balen Adam

Leeds Fertility

Background: Time lapse imaging incubators have been shown to improve embryological and clinical outcomes following IVF. It has been unclear as to whether this is due to the uninterrupted culture system that it provides or better embryo selection.

Methods: Single step culture medium was used for embryo culture in both time lapse imaging incubators and standard culture incubators (benchtop and chamber incubators) from November 2015 at our unit. From November 2015 to December 2017, the embryos in standard culture were removed for Day 3 morphological assessment (Standard culture interrupted). From January 2018, this was no longer performed and embryos were undisturbed until Day 5, unless there was only one zygote available (Standard culture uninterrupted).

Results: The embryo utilisation rate (Number of embryos transferred or cryopreserved per 100 zygotes) was significantly higher in the standard culture uninterrupted arm than the standard culture interrupted arm (39.8% vs 33.6%, Risk Ratio (RR) 1.1856, 95% CI 1.1159 to 1.2596). It was noted that use of the uninterrupted system in standard culture raised the utilisation rate to parity with the time lapse imaging incubator system (39.8% vs 38.8%, RR 1.0249, 95% CI 0.9781 to 1.0739).

Conclusion: To optimise the benefit of a single step culture medium, interruptions to culture for Day 3 morphological assessment should be avoided. For clinical settings where this is inappropriate, use of time lapse imaging systems might provide a useful alternative.

P202 Is day three KIDScore an effective tool to predict blastocyst development, and defer embryo transfer to day five?

Hardy Chloe¹; Harbottle Stephen¹; Carroll Michael²; Heywood Katie¹; Greer Katherine¹

¹Cambridge IVF, Kefford House; ²Manchester Metropolitan University

Background: Embryos are usually incubated until the third day (D3) of development, when quality is assessed based on appearance. Patients with ≥ 3 good embryos are advised to delay Embryo Transfer (ET) until day five (D5). D5 Blastocyst transfer is preferable as pregnancy rate is higher¹. Delaying embryo transfer may result in no ET, as some embryos stop developing between D3 and D5. Patients with lower numbers of embryos are currently advised to have D3 ET. Embryoscope uses time-lapse morphokinetic data to calculate a Known Implantation Data Score (KIDScore) based on the timing of developmental events². KIDScore ranges from 1-5; a higher score identifying embryos with an increased implantation potential. Aim: Can D3 KIDScore predict useable blastocyst development by D5, in order to defer ET?

Methods: A retrospective data analysis of embryos cultured in the EmbryoScope® until at least D5; between January 2017 and December 2018. Embryos were assigned a morphology grade and KIDScore at 66 hours post insemination (PI) (D3) and 114-120 hours PI (D5). D5 Blastocysts graded $\geq 2Bb$ were classed as useable. Three predictive models were proposed; D3 KIDScore, morphology and KIDScore as an adjunct to morphology. Standard covariates included patient age and treatment type (IVF/ICSI). Receiver operating characteristic (ROC) curves were generated to compare the predictive strength of each model.

Results: 842 embryos from 148 patients were included. ROC analysis showed KIDScore (AUC: 0.786, 95%CI 0.754-0.818, PPV:72.6, NPV:74.5) and conventional morphology (AUC: 0.780, 95%CI 0.748-0.811; PPV:69.1, NPV:76.5) were fair predictive models. When KIDScore was used as an adjunct to morphology the predictive capability increased (AUC: 0.823 95%CI 0.795-0.852; PPV:72.5, NPV:76.7).

Conclusion: These findings suggest D3 KIDScore can be used to predict blastocyst development, particularly when adjunct to conventional morphology. The model can be used to defer ET to D5, even when embryo number is low.

1. Glujovsky et al. (2016) Cochrane Database Syst Rev, CD002118; Maurer et al. (2015) Int. J. Fertility and Sterility, 9(3) pp. 346- 353 2. Petersen et al. (2016) Human Reproduction, 31(10) pp. 2231-2244

P203 Preliminary experience with KIDScore

Smale Daniela; Neville Connie; Gunn Joel; Price Anthony; Ingamells Sue

Wessex Fertility

Background: Although single time point assessment of embryo morphology has been the traditional method for embryo selection, it often misses significant developmental events. Time lapse imaging has facilitated the use of morphokinetic algorithms to successfully predict embryo viability[1]. The aim of this study was to validate Vitrolife's Day 5 Known implantation data score (KIDScore) using retrospective pregnancy data from frozen embryo transfer (FET) data.

Methods: Descendent blastocyst FET data was collected from fresh IVF/ICSI cycles performed between January 2018 and June 2019. Double FET cycles were excluded. A random selection of grade A (n=45), C (n=40) and D (n=35) blastocysts were annotated using the KIDScore algorithm and split into four groups K1-4 (1=<4, 2=4-5.9, 3=6-7.9 and 4=8-10). The groups were compared using implantation, clinical pregnancy and miscarriage rates as outcome measures. Additionally, the proportion of Grade A, C and D embryos were compared between each group. Statistical analysis was carried out using the Kruskal-Wallis and Mann-Whitney tests.

Results: Implantation rate appeared to vary according to KIDScore with significant differences noted between K1/K3 (41.5%/73.85% p=0.002), K1/K4 (41.5%/84.94% p=<0.0001) and K2/K4 (59.76/84.94 p=0.0063). There was no significant difference in clinical pregnancy or miscarriage rate between groups. The proportion of Grade A blastocysts progressively increased from K1-K4 (5-70%) while the proportion of Grade D blastocysts showed a progressive decrease from K1-K4 (63-11%). No trends were noted with grade C Blastocysts.

Discussion: These results suggest that the KIDScore gives a reliable indication of blastocyst implantation potential but is less indicative of clinical pregnancy and miscarriage. The uniform distribution of grade C and presence of grade D embryos in each group suggests morphological grading alone may exclude viable embryos from transfer and cryopreservation.

Conclusion: This study supports the use of KIDScore to aid embryo selection and may be superior to morphological grading alone.

1. Adolfsson E, Porath S and Andershed A. External validation of a time-lapse model; a retrospective study comparing embryo evaluation using a morphokinetic model to standard morphology with live birth as endpoint. JBRA Assist Reprod. 2018 Jul-Sept 22(3): 205-214.

P204 Embryo development and function is compromised by concentrations of advanced glycation end products found within the obese uterine environment

Hutchison Jennifer¹; Truong Thi Thanhmai²; Salomonsen Lois³; Evans Jemma³; Gardner David²

¹Department of Molecular and Translational Science; Centre for Reproductive Health; ²School of Biosciences, University of Melbourne, Australia; ³Centre for Reproductive Health, Hudson Institute of Medical Research, Melbourne, Australia

Background: Obesity is a global pandemic: In England, 64% of the population is overweight or obese, and critically ~30% of women of reproductive age are obese (NHS Digital). Obesity reduces natural and IVF pregnancy success, and increases risk of miscarriage & preeclampsia. 'Toxic' Advanced Glycation End Products (AGEs), formed when reducing sugars react with the free amino group on proteins, are elevated four-fold in the uterine cavity of obese, infertile women versus lean.

Aim: To examine whether AGE concentrations physiologically representative of the obese uterine environment impact preimplantation embryo development and function.

Method: Preimplantation mouse embryos were cultured with AGEs equimolar with the uterine environment of lean (1 µM) versus obese (8 µM) women. 1) AGEs receptor (RAGE & TLR4) immunolocalisation, & TUNEL staining (apoptosis assessment), investigated potential mechanisms of AGE action; 2) Developmental morphokinetics assessed by time-lapse microscopy (Embryoscope, Vitrolife); 3) Trophoctoderm and inner cell mass cellular allocation determined by differential staining; 4) Implantation potential assessed by blastocyst outgrowth assay; 5) Therapeutics (RAGE antagonist, metformin, and antioxidant cocktail) investigated for potential to ameliorate AGE-mediated effects.

Results: Preimplantation embryos express RAGE & TLR4, providing a mechanism for AGE-mediated signaling. "Obese" AGEs significantly impacted embryo development and function: 1) Developmental delay as demonstrated by morphokinetics (~3 hour lag). 2) Reduction in proliferation as indicated by reduced total cell number (23%, p<0.0001) but no effect on apoptosis. 3) Implantation potential compromised: reduced hatching (25%, p<0.001), trophoctoderm

cell number (23%, $p < 0.001$), and reduced outgrowth (~30%, $p < 0.01$). Embryo development was somewhat improved by RAGE antagonism.

Conclusion: Preimplantation development occurs within the uterine cavity, an environment which has the potential to influence embryonic development and pregnancy outcomes. Elevated uterine AGEs detrimentally impact multiple aspects of preimplantation embryo development providing a critical link between obesity and reduced fertility. Preconception reduction of AGEs may improve fertility outcomes of obese women.

P205 Do we need a laser to perform trophectoderm biopsy for PGT-A?

Armstrong Ellen; Campbell Alison

CARE Fertility

Aim: To determine if the use of the laser at the point of severing cells for genetic testing improves outcome compared to mechanical biopsy only.

Methodology: 5813 blastocysts which underwent a trophectoderm biopsy on Day 5 or Day 6 of culture were retrospectively analysed for PGT-A results. 1062 blastocysts were warmed for treatment and were then assessed for survival and for biochemical and clinical pregnancy outcomes. Statistical analysis was performed using Fishers exact 2 tailed test with a significant p value of < 0.05 .

Results: 4182 blastocysts were biopsied using two 4.8µm laser oblations to weaken the gap junctions, followed by quick mechanical movement ('flick') of the biopsy pipette against the holding pipette to separate the sample for testing to infer blastocyst ploidy. The remaining 1631 blastocysts were biopsied using the mechanical method only, without the laser. No other differences in laboratory protocols were present. Comparing laser and mechanical, non significant differences ($p > 0.05$) were seen in successful amplification rates (95.6% and 96.4% respectively), biochemical (64.4% and 57.4% respectively) and clinical pregnancy outcomes (50.3% and 45.1% respectively). Significance ($p = 0.0273$) was seen in blastocyst survival post thaw with blastocysts that had been biopsied using the laser ($n = 771$, 97.8%), surviving at a higher rate compared to those mechanically biopsied only ($n = 294$, 94.8%).

Conclusions: This large retrospective multicentre analysis of biopsied blastocysts has demonstrated that a laser does not significantly improve amplification and pregnancy outcome, but may aid survival rate post warming. A larger study is required to confirm the result regarding survival rate due to the disproportionate group numbers.

P206 Effects of AdipoRon, an adiponectin receptor agonist, on proliferation and steroid secretion by human granulosa cells

Grandhaye Jérémy¹; Estienne Anthony¹; Hmadeh Sandy²; Plotton Ingrid³; Levasseur Floriane¹; Remy LeGuevel⁴; Le Vern Yves⁵; Ramé Christelle¹; Jeanpierre Eric¹; Guerif Fabrice¹; Dupont Joëlle¹; Froment Pascal¹

¹INRA UMR85 Physiologie de la Reproduction et des Comportements; ²Strasbourg University; ³Molecular Endocrinology and Rare Diseases, University Hospital, Claude Bernard Lyon 1 University; ⁴ImPacell platform, SFR BIOSIT, University of Rennes1; ⁵INRA UMR1282 Infectiologie et Santé Publique, Cytometry Platform

Metabolic disturbance such as insulin resistance or fatness can alter the ovarian activity. Adipose tissue can impact directly fertility through the hundreds of cytokines produced.. One of them is adiponectin, a cytokine used as a marker of fatness and inflammation. In vitro, our team has observed that adiponectin modulated steroid secretion by ovarian granulosa cells. The adiponectin concentration in the follicular fluid is associated with the metabolic status. Recently, an adiponectin mimetic drug called 'AdipoRon' has been developed and present a relevant interest in the ovarian control. The aim of our study was to determine the role of AdipoRon on the human granulosa cells activity.

The experiments were performed on human granulosa cell line (KGN) (1), and human primary granulosa cells prepared from IVF protocol. The cells were exposed to AdipoRon (2.5 µM and 25 µM) for 48h. The 25 µM concentration has been described in the literature to activate strongly the adiponectin pathway (2).

After 48 hours of AdipoRon (25 μ M) exposure, KGN showed a 18% reduction in cell proliferation ($p < 0.01$) and 4% for primary granulosa cells ($p < 0.05$) determined by BrDU incorporation and PCNA protein expression, a marker of S Phase of the cell cycle. Flow cytometry have shown a significant accumulation of cells in G1 phase when treated with 25 μ M of AdipoRon ($p < 0.001$). AdipoRon modified cell metabolism with an increase in lactate production ($p < 0.05$), and reduction in steroid synthesis (enzymes: 3β HSD, $p < 0.001$ and aromatase, $p < 0.01$) and secretion (estradiol, $p < 0.05$, testosterone, $p < 0.05$).

In conclusion, these results indicate that AdipoRon is able to modify the metabolism of granulosa cells and could be used in the development of new therapeutic strategies requiring the reduction in ovarian cell proliferation or steroid production.

1. Nishi Y, Yanase T, Mu Y, Oba K, Ichino I, Saito M, et al. Establishment and characterization of a steroidogenic human granulosa-like tumor cell line, KGN, that expresses functional follicle-stimulating hormone receptor. *Endocrinology*. 2001 Jan;142(1):437–45.

2. Fairaq A, Shawky NM, Osman I, Pichavaram P, Segar L. AdipoRon, an adiponectin receptor agonist, attenuates PDGF-induced VSMC proliferation through inhibition of mTOR signaling independent of AMPK: Implications toward suppression of neointimal hyperplasia. *Pharmacological Research*. 2017 May 1;119:289–302.

P207 Naringenin upregulates the expression of CYP17 and CYP 19 in the rats' testes; any effects on sperm functionality?

Adana Misturah¹; Akang Edidiong²; Eche Simeon³; Ugochukwu Offor³; Azu Onyemaechi³

¹University of Ilorin; ²University of Lagos; ³University of KwaZulu-Natal

Background: CYP17A1 and CYP19A1, are members of the cytochrome P450 superfamily which are monooxygenases that catalyze many reactions involved in steroidogenesis. In humans, the gene CYP17, located on chromosome 10q24.3, encodes the 17 α -hydroxylase while CYP19 gene which resides on chromosome 15q21.1 encodes aromatase enzyme. They are both expressed in the gonads. Optimal spermatogenesis has been shown to be dependent on the expression of these genes. They are known to be crucial for germ cell development and functionality. This research studied the expression of these genes in the rats' testes and investigated the effects of Naringenin, a bioactive flavonoid on their expression.

Methods: Thirty male rats weighing 200-220g, were randomly assigned into 3 treatment groups- DW: Distilled water, N40: Naringenin, 40 mg/kg, N80: Naringenin, 80 mg/kg. Treatment lasted for a period of 70 days. Rats were then sacrificed, blood samples collected for hormonal assay, testes were harvested and semen were analysed. Expressions of CYP 17 and CYP 19 genes were done via real-time PCR. The Animal Research Ethical Committee, UKZN, South Africa approved this research (reference: AREC/046/016D).

Results: The animals in groups N40 and N80 displayed significantly higher expression of CYP 17 and CYP 19 genes when compared to controls. Serum testosterone and LH levels were also significantly higher. However, no significant differences in the sperm count and percentage of abnormal sperms observed across the groups. But there were significantly lower progressive sperms in group N40 when compared to control.

Conclusion: The study suggests that even though Naringenin displayed increased expression of CYP 17 and CYP19 genes in the testes, there was no significant impact on the semen parameters and no effects on the overall testicular function. Naringenin, a bioflavonoid may be able to protect against testicular toxicity but does not improve the function of an otherwise healthy testes.

P208 Time lapse and older patients; does it improve their chances?

Freeman Charlene; Blower Jane; Gelbya Tarek; Sharpe Amy; Potdar Neelam

Leicester Fertility Centre

Many IVF clinics within the UK are using undisturbed embryo culture and time Lapse imaging techniques to monitor embryo development. It is postulated that this may lead to improved embryo quality and increased utilisation. This is a

retrospective study comparing embryo utilisation rates and clinical pregnancy rates of patients <35 and those ≥35 using conventional big box incubators (6% CO₂ in air) versus those using a time lapse incubation system (6% CO₂, 5% O₂) (GeriTM) Patients undergoing IVF/ICSI using their own eggs and receiving fresh embryo transfer between 01/01/13 until 16/08/19 were included in this study. Egg sharers and patients split between incubators were not included in the study Clinical pregnancy was determined by the presence of a foetal heart at 7 week scan. Negative results were those with a negative pregnancy test, no FH at scan, ectopic. Patients aged <35: Big box incubator n= 460 GeriTM n=244 total: 704 Patients aged ≥35: Big box incubator n= 302 GeriTM n=231 total: 533 The data showed statistically significant increase in clinical pregnancy rates for patients <35 years using the time lapse system (p=0.0661), however the results for patients ≥35 years was not statistically significant (p=0.1593). Both groups showed a move towards extended culture. Patients obtaining embryos suitable for cryopreservation was significantly increased for those <35 in the GeriTM (p=0.0001) compared to that of the big box incubator. Those aged ≥35 showed some increase but not enough to be statistically significant at this time (p=0.0985). In conclusion, the move towards undisturbed culture in time lapse incubators seems to increase embryo utilisation and positive outcomes for those <35. Those aged ≥35 and over did not seem to benefit as much. However, as the numbers are still small, more information is needed to better understand the findings and utilise this technology to benefit patients.

GERI Time lapse incubators MERCK Copyright 2019 Merck KGaA, Darmstadt, Germany

P209 Species-specific dynamics of mitochondrial content within an embryo during preimplantation development in mice and cattle

Komatsu Masaya; Yokoi Fumi; Imai Shun; Yamane Maiko; Bai Hanako; Takahashi Masashi; Kawahara Manabu

Hokkaido University

Introduction: Mitochondria play essential roles in eukaryotes; for example, ATP production and regulation of apoptosis. Therefore, it has been believed that the mitochondrial content within an embryo significantly affect embryonic development in mammals. However, there have been no studies to compare the dynamics of mitochondrial content between different species during preimplantation development by the identical experimental system. In this study, we investigated the number of mitochondria in both mouse and bovine embryos by measuring the copy number of mitochondrial DNA (mtDNA). Furthermore, we analyzed mRNA expression of mtDNA-replication-related genes.

Methods: Mouse and bovine preimplantation embryos were prepared by in vitro fertilization (IVF). Total DNAs derived from individual IVF embryo were collected for analysis of mtDNA copy number (n = 20 in each stage). Total RNAs were also collected from mouse and bovine embryos for the quantitative PCR (qPCR) analysis. To examine the mtDNA-replication-related gene expression, the mRNA expression levels of 8 genes; Pop5, Ssbp1, PolgA, PolgB, Nrf1, Tfam, Tfb1m, and Tfb2m, were examined at the 4-cell and 8-cell stages in mice, and at the 8-cell, 16-cell, morula, and blastocyst stages in cattle.

Results: In mouse embryos, the major increase in mtDNA copy number was observed once during preimplantation development, which occurred from the 4-cell (151,430 ± 47,629 copies) to the 8-cell stages (924,600 ± 184,414 copies). Whereas, the mtDNA of bovine embryos was increased in two steps, which were observed from the 8-cell (954,667 ± 77,369 copies) to the 16-cell (3,977,000 ± 759,048 copies) stages, and thereafter, from the morula (4,316,000 ± 759,664 copies) to the blastocyst (9,738,000 ± 1,498,225 copies) stages. At these mtDNA-increasing stages, we confirmed the expression of mtDNA-replication-related genes in both mouse and bovine embryos.

Conclusion: Our results indicated that mtDNA replication during preimplantation development was regulated in species-specific manner.

P210 Impact of below average trophectoderm or inner cell mass grading on implantation rates in 2385 consecutive cases

Moreira Catarina; Shah Trina; Linara-Demakakou Elena; Ahuja Kamal; Macklon Nick; Wang Jinjun

London Women's Clinic - London

Background/Purpose: Some studies have demonstrated greater importance of trophoctoderm (TE) versus inner cell mass (ICM) grades in determining implantation rates (IR) ^[1]. This study looked at the impact of the below average TE with good quality ICM and below average ICM with good TE blastocysts on IR.

Methods: Retrospective analysis of 2385 single blastocyst transfers performed between January 2016 and June 2019. ICM and TE were scored as A-B-C-D ^[2,3]. Category D was for degenerative cells ^[2]. From these transfers, 853 were fresh (n=700 4BB, 122 4BC, 18 4CB, 13 4CC blastocysts) and 1532 were frozen (n=1040 4BB, 381 4BC, 50 4CB, 61 4CC). Analysis included expanded blastocysts (grade 4) created with all age groups and both own and donor, fresh and frozen, eggs. Data was analysed by egg age. Study excluded blastocyst biopsy cases. The main outcome measure was IR.

Results: IR of 4BB, 4BC, 4CB, 4CC blastocysts were respectively as follows 44%, 33%, 39%, 31% in fresh cycles; 40%, 29%, 34%, 20% in frozen cycles; and 42%, 30%, 35%, 22% in total fresh and frozen cycles. The mean ages of patients in different categories of blastocysts were comparable 4BB - 33.9 years; 4BC - 35.1 years; 4CB - 35.6 years; 4CC - 34.9 years. IR between groups 4BB versus 4BC and 4BB versus 4CC were statistically different (p<0.05) but not statistically different between 4BB and 4CB.

Conclusion: Higher IR was seen when single embryo transfer (SET) performed with 4CB rather than with 4BC or 4CC blastocysts, indicating that blastocysts with better quality TE achieve greater outcomes than better quality ICM even when ICM is below average quality. Our analysis also provides evidence in favour of continuing with SET involving 4CB fresh and frozen blastocysts.

1. Hill MJ, Richter KS, Heitmann RJ, Graham JR, Tucker MJ, DeCherney AH, et al. Trophoctoderm grade predicts outcomes of single-blastocyst transfers. *Fertil Steril*. 2013 Apr;99(5):1283-1289.

2. Veeck L, Zaninovic N. *An Atlas of Human Blastocysts*. New York, London: Informa Healthcare; 2003.

3. Gardner DK, Schoolcraft WB, Jansen R, Mortimer D. *In vitro culture of human blastocysts. Toward Reproductive Certainty: Fertility and Genetics Beyond 1999*; 1999 UK Parthenon Publishing London:378-388.

P211 Aetiology and prevalence of uterine factor abnormality among women attending infertility clinic at a tertiary hospital in north-west Nigeria: a cross-sectional study

Popoola Abdulrafiu¹; Adesiyun Adebisi²

¹Nigeria Defence Academy Hospital, Kaduna; ²Ahmadu Bello University Teaching Hospital

Statement of problem: Infertility is a common gynaecological problem in Nigeria. The impact of infertility goes beyond childlessness and the woman bears the psychosocial consequences. An abnormal uterus in the severe form poses an almost insurmountable challenge to procreation in the developing world because of late presentation to treatment which are mainly surgical and foregoing issues with accessibility to assisted reproductive technology

Objective: This study determined the prevalence and causes of uterine abnormalities in women with infertility .

Methodology: A cross-sectional study. All patients had pelvic sonogram and hysteroqram. Hysteroscopy with or without laparoscopy was done where indicated.

Results: A total of 350 infertile women were studied. The prevalence of uterine factor infertility was 48%(n=169). Of the 169 cases, 163(96.4%) and 6(3.6%) had acquired and congenital uterine abnormalities respectively. Uterine fibroid(60.9%) and Asherman's syndrome(28.4%) accounted for the majority of uterine factor abnormality encountered in this study.

Conclusion: The burden of uterine factor recorded in this study is high. Acquired uterine factors are far more the commonest and uterine fibroid top the list. Institution of yearly policy of ultrasonographic surveillance for uterine fibroid in women of reproductive age would be the right way to go towards early detection and management. Promulgation of policies and strategies to prevent unsafe abortion would help to decrease the incidence of Asherman's syndrome which is the second commonly encountered uterine factor abnormality in this study. Prevention is paramount because surrogacy assisted conception treatment and the upcoming treatment with uterine transplantation remains a rarity in the developing world.

P212 Clinical outcome of day 6 cryopreserved embryos transferred into day 5 endometrium

White Linsey; Leitch Joanne; Collins Daniel; Beaton Catherine

Glasgow Royal Infirmary

Aims/Objectives: Elective single embryo transfer (eSET) has been adopted in UK clinics to reduce multiple pregnancies. In turn, this has led to culturing embryos to day 5 in single-step media for blastocyst transfer, and increased embryos identified for vitrification. Due to reported success rates with vitrification/warming, Glasgow Royal Infirmary (GRI) introduced vitrification of blastocysts Feb2016 (day 5 & day 6), as opposed to slow freezing. Clinical outcome data of day 5 and day 6 warmed and transferred into day 5 endometrium has taken place.

Content: Data was collected 2016-2018 following revised protocol for vitrification/warming of embryos. This was analysed to determine if clinical outcomes were favourable using new method and also if there was a difference between clinical outcomes of day 5 or day 6 transfer into a day 5 endometrium. Multiple pregnancy rate was reviewed to determine if there is a difference between implantation rate of day 5 and day 6 embryos.

Outcomes: Statistical analysis showed there was a significant difference ($p < 0.001$ $n=1476$) after introducing the revised vitrification and warming protocols with increased clinical outcomes increasing by 13%. Ongoing/live birth outcomes for day 5 and day 6 frozen embryo transfers showed no significant difference ($p=0.82$ $n=435$), reported at 39% and 40% respectively. Statistical analysis ($p=0.95$ $n=435$) indicated there was no difference in clinical outcome of implantation rate of day 5 and day 6 embryos transferred into day 5 endometrium.

Discussion: Since revised vitrification and warming protocols were introduced at the GRI a significant increase in frozen clinical outcomes was observed. No significant difference in clinical outcome or implantation rate between warmed day 5 and day 6 embryos transferred into a day 5 endometrium was observed. A total of 39 pregnancies were

P213 Embryo-secreted microRNA miR-294 is correlated to DNA fragmentation at the blastocyst stage

Makri Dimitra; Michailidou Eftychia; Efthymiou Panagiota; Maalouf Walid

University of Nottingham

Purpose/background: MicroRNAs are small non-coding RNA molecules with important regulatory actions in gene expression acting mainly via RNA silencing and post-transcriptional changes. They are involved in numerous cell processes such as signaling, cycle progression, and apoptosis. It is well known that increased apoptosis at the blastocyst stage impairs the implantation potential of the embryo and affects pregnancy rates. During embryo development, microRNAs are released in the spent culture medium (SCM) and these are potentially useful biomarkers of aneuploidy and implantation in humans. However, there are no reports about the value of microRNA analysis of SCM collected from individually cultured embryos in regards to extent of embryonic apoptosis.

Methods: Frozen mouse zygotes (B6C3F-1 x B6D2F-1) ($N=70$) were thawed and cultured in individual drops of culture medium at 37°C, 5% CO₂ to the Expanded Blastocyst stage in two consecutive repetitions. The blastocysts were scored using a morphology grading system assessing the trophectoderm and inner cell mass quality. SCM samples ($N=59$) were collected in minimum volume (20µl) and analysed for the presence of miR-294, a key apoptosis regulator, using the polymerase chain reaction method (PCR). The collected blastocysts were assessed for apoptosis using the TdT-mediated dUTP-X nick end labelling method (TUNEL). MiR-294 levels were compared to the apoptotic extent of each embryo to identify correlations.

Results: After blastocyst scoring, the embryos were further categorised in Good, Fair, and Poor morphology groups. From the 59 blastocysts, 4 were of excellent quality (Good), 37 were Fair, and 6 were Poor morphology embryos. The average apoptotic percentage was 12% ($N=53$). Mean comparisons between the 3 morphology groups showed that Good, Fair, and Poor morphology embryos have similar extent of apoptosis ($P > 0.05$), meaning that excellent morphology is not always associated with good DNA quality and thus morphological scoring is not a valuable predictor of cellular quality

P214 Could the use of TUDCA in in vitro culture of bovine embryos be beneficial to its development?

Mariano Pioltine Elisa; Bortoliero Costa Camila; Gouveia Nogueira Marcelo Fábio

São Paulo State University (UNESP)

Expose oocytes and embryos to manipulation under in vitro conditions cause a variety of cellular stresses that decrease the embryonic development competence [1]. Endoplasmic reticulum (ER) stress, in turn, leads to the activation of a series of adaptive pathways known as unfolded protein response (UPR) to maintain ER homeostasis [2]. In cases where ER stress is prolonged or too severe to resolve, apoptosis is induced [3]. Tauroursodeoxycholic acid (TUDCA), which is a bile acid that acts as a potent chemical chaperone to inhibit ER stress in vitro [4], has been widely used during in vitro maturation of oocyte and/or embryo development. The aim of this study was to evaluate the effect of three increasing concentrations of TUDCA (Control; 50 μ M - T50; 200 μ M - T200; 1,000 μ M - T1000) in an in vitro culture with low oxygen tension (5%), on bovine blastocyst rate and hatching kinetics. Evaluations were performed on days 7, 8 and 9 after fertilization step. Five replicates were performed with 45 probable zygotes/group. The effect of treatments was tested by one way-ANOVA and the means were compared with Tukey Test. Differences were considered significant when $P \leq 0.05$. On day 7, the T1000 group (33.1%) was statistically different from the T200 group (43.5%) when the blastocyst rate was assessed. In embryo hatching kinetics, there was no difference between treatments on day 7. On days 8 and 9, the T1000 group (7.3% and 6.95%, respectively) was statistically different from day 8 Control (26.4%) and all treatments by day 9 (23.8%, 24.2% and 27.9%; respectively to Control, T50 and T200). The results suggest a toxic effect in the group treated with 1,000 μ M of TUDCA altering the embryonic development and kinetics. In addition, treatment with 200 μ M TUDCA appears to be positive for the embryo, even in low oxygen system.

1. Song BS, Yoon SB, Kim JS, Sim BW, Kim YH, Cha JJ et al. Induction of autophagy promotes pre attachment development of bovine embryos by reducing endoplasmic reticulum stress. *Biol. Reprod.* 2012; 87(1):8, 1-11. 2. Boyce M, Yuan J. Cellular response to endoplasmic reticulum stress: a matter of life or death. *Cell Death Differ.* 2006; 13: 363-373. 3. Tabas I, Ron D. Integrating the mechanisms of apoptosis induced by endoplasmic reticulum stress. *Nat Cell Biol.* 2011; 13: 184-190. 4. Xie Q, Khaoustov VI, Chung CC, Sohn J, Krishnan B, Lewis DE et al. Effect of tauroursodeoxycholic acid on endoplasmic reticulum stress-induced caspase-12 activation. *Hepatology.* 2002; 36, 592-601.

P215 Optimal concentration of Acetyl-L Carnitine for use in in vitro bovine embryo production

Güven Ates Gizem; Tutt Desmond AR; Kwong Wing-Yee; Clare Constance E; Sinclair Kevin D

School of Biosciences, University of Nottingham, UK

Background: Fatty acid metabolism by oocytes and pre-implantation embryos is required to support early development, but the timing of this process and how it is regulated are poorly understood [1]. The β -oxidation of fatty acids by mitochondria is enhanced when L-carnitine is added to media, leading to improvements in embryo development [2,3,4]. Mechanisms of action of the acetyl-ester of L-carnitine (ALC), are less well understood, although antioxidant and regulator effects on mitochondrial metabolism have been reported [5,6]. As a more bioactive form of carnitine, uncertainty also surrounds the optimal concentration (μ M to mM) of ALC for use in in vitro embryo production. This aspect was addressed in the current study.

Method: Abattoir-derived bovine oocytes were matured (IVM) and zygotes cultured (IVC) in the presence of ALC at 0, 10, 100 and 1000 μ M in an experiment replicated 10 times. Cleavage and Day 8 stage morphological data were collected and cell number and allocation to either the inner-cell mass or trophectoderm assessed by immunocytochemistry for the first six replicates. Oocytes and blastocysts from the remaining four replicates were frozen for lipid analyses by GC-MS. Proportions were analysed by logistic regression assuming binomial errors and count data assumed Poisson errors.

Result: There was no effect of ALC on proportion cleaved of inseminated (0.715 ± 0.0148), proportion blastocysts of inseminated (0.378 ± 0.0134) or of cleaved (0.528 ± 0.0167). However, blastocyst cell number decreased ($P = 0.011$) with doses of ALC $> 10 \mu$ M (130.3 ± 6.29 , 123.5 ± 8.89 , 100.8 ± 7.96 and 101.8 ± 8.39 cells for 0, 10, 100 and 1000 μ M respectively).

Conclusion: Putative beneficial effects of ALC may be lost in the high μM to mM range; higher concentrations may be cytotoxic. This conclusion, however, awaits ongoing analyses of lipid metabolism and molecular aspects of mitochondrial regulation.

1. Dunning KR and Robker RL. Promoting lipid utilization with L-carnitine to improve oocyte quality. *Anim Reprod Sci* 2012; 134: 69-75
2. Dunning KR, Cashman K, Russell DL, Thompson JG, Norman RJ and Robker RL. Beta-Oxidation Is Essential for Mouse Oocyte Developmental Competence and Early Embryo Development. *Biology of Reproduction* 2010; 83: 909-918
3. Wu GQ, Jia BY, Li JJ, Fu XW, Zhou GB, Hou YP and Zhu SE. L-carnitine enhances oocyte maturation and development of parthenogenetic embryos in pigs. *Theriogenology* 2011; 76:785-793.
4. Phongnimitr T, Liang Y, Srirattana K, Panyawai K, Sripunya N, Treetampinich C, Parnpai R. Effect of L-carnitine on maturation, cryo-tolerance and embryo developmental competence of bovine oocytes. *Anim Sci J*. 2013; 84:719-725.
5. Truong T and Gardner DK. Antioxidants improve IVF outcome and subsequent embryo development in the mouse. *Human Reproduction* 2017; 32: 2404-2413.
6. Xu HY, Yang XG, Lu SS, Liang XW, Lu YQ, Zhang M and Lu KH.. Treatment with acetyl-L-carnitine during in vitro maturation of buffalo oocytes improves oocyte quality and subsequent embryonic development *Theriogenology* 2018; 118: 80-89.

P216 Effects of high non-esterified fatty acids exclusively during in vitro fertilisation on levels of reactive oxygen species in bovine zygotes

Idriss Abdullah¹; Okello Edward¹; Sturmey Roger²; Velazquez Miguel¹

¹Newcastle University; ²Hull York Medical School

High non-esterified fatty acids (NEFA) levels present in obese individuals and in cows experiencing negative energy balance has been linked with impaired oocyte developmental competence. Cattle models have demonstrated that in vitro exposure to NEFA (i.e. stearic acid [SA], palmitic acid [PA], oleic acid [OA]) at high concentrations during oocyte maturation and embryo development can disrupt both embryo formation and quality [1-2]. However, less is known about the effects of high NEFA during the fertilisation process per se [3]. The aim of this study was to determine the effects of high NEFA exclusively during bovine fertilisation in vitro on the levels of reactive oxygen species (ROS) in the resultant zygotes. This information could provide knowledge on potential therapies to ameliorate NEFA toxicity during the periconceptional period. In vitro matured oocytes and spermatozoa were incubated for 19 h with different NEFA-enriched environment (4 replicates) corresponding to physiological (Control-1, 28 μM SA, 23 μM PA, 21 μM OA; n=128) and pathophysiological (High-NEFA, 280 μM SA, 230 μM PA, 210 μM OA; n=131) relevant concentrations [3]. A third group contained solvent (Control-2, n=98). Pronuclei were stained with Hoechst (Sigma, UK) and ROS levels (CellROX™ molecular probes, UK) were determined with epifluorescence microscopy only in zygotes. Fluorescence intensity was measured with the ZEISS ZEN 2.6 software. Data were analysed by ANOVA. Fertilisation rate (Control-1=65%, Control-2=70%, High NEFA=47%) and level of polyspermy (Control-1=16%, Control-2=17%, High NEFA=9%) were not affected by treatment. Similarly, NEFA levels did not affect ROS levels in zygotes. In conclusion, under the conditions of the present study, high exposure to NEFA during bovine IVF did not alter ROS levels in the resultant zygotes.

- [1] Desmet KL, Van Hoeck V, Gagné D, Fournier E, Thakur A, O'Doherty AM, Walsh CP, Sirard MA, Bols PE, Leroy JL. 2016. Exposure of bovine oocytes and embryos to elevated non-esterified fatty acid concentrations: integration of epigenetic and transcriptomic signatures in resultant blastocysts. *BMC Genomics* 17(1):1004. [2] Van Hoeck V, Leroy JL, Arias Alvarez M, Rizos D, Gutierrez-Adan A, Schnorbusch K, Bols PE, Leese HJ, Sturmey RG. 2013. Oocyte developmental failure in response to elevated nonesterified fatty acid concentrations: mechanistic insights. *Reproduction* 145(1):33-44. [3] Desmet KLJ, Marei WFA, Pintelon I, Bols PEJ, Leroy JLMR. 2018. The effect of elevated non-esterified fatty acid concentrations on bovine spermatozoa and on oocyte in vitro fertilisation. *Reprod Fertil Dev* 30(11):1553-1565.

P217 Non-invasive embryo evaluation and selection in Assisted Reproduction Treatment (ART): Systematic review and meta-analysis

Aquilina Marie Claire¹; Maalouf Walid¹; Jayaprakasan Kannamannadiar²

¹University of Nottingham, UK; ²University of Nottingham, UK and Royal Derby Hospital, Derby, UK

Background: The safety, clinical and cost-effectiveness of time-lapse systems (TLS) relative to conventional incubation have been evaluated in a number of controlled studies and a few systematic reviews. However, the benefit of TLS for single embryo transfer at different embryo developmental stage has not been reviewed.

Methods: PRISMA guidelines were followed to systematically identify, review and meta-analyse the literature that compared time-lapse embryo incubation and conventional incubation to ART outcomes. Electronic databases (OVID Medline, Embase and Cochrane Central Register of Controlled Trials) up to July 2019 were searched. 16 studies met the inclusion criteria. The data was analysed using RevMan v-5.3 to calculate Mantel-Haenszel Odds ratio (ORs) and 95% confidence interval (CI) and to examine heterogeneity. Study quality was assessed using GRADE criteria for randomised controlled trials and "Newcastle-Ottawa scale" for non-randomised controlled studies. The primary outcome was live birth/ongoing pregnancy rates. Clinical pregnancy rates, early pregnancy loss, implantation rates were also assessed.

Results: TLS for blastocyst SET indicated similar live birth/ongoing pregnancy rates (OR 1.01 [95%CI 0.65-1.58]) and early pregnancy loss (OR 1.03 [95%CI 0.32-3.32]). Similar findings were observed for TLS for cleavage stage SETs. The pooled data (combining the data for transfer of single or double or multiple blastocyst and cleavage stage embryos) showed significantly increased livebirth/ongoing pregnancy rate (OR 1.22 [95%CI 1.05-1.41]) and decreased early pregnancy loss (OR 0.71 [95%CI 0.54-0.93]) associated with TLS relative to standard incubation.

Discussion: While there is no evidence to show a clear benefit of TLS for single cleavage stage or blastocyst stage embryo transfer, the pooled data showed significant improvements in clinical outcomes associated with TLS. Clinics performing cleavage or blastocyst stage embryo transfer can utilise the data generated in this study to counsel women/couple undergoing IVF and thereby assisting them to make an informed decision.

P218 Examining the effect of nicotine on a mouse model of the ovarian reserve

Idrees Sara; Fenwick Mark

University of Sheffield

Background: Smoking is considered one of the major lifestyle factors that can affect ovarian follicle development, reproductive health and fertility. Over the past decade, there has been a dramatic shift in the number of people who have exchanged cigarette smoking for less harmful alternatives such as electronic cigarettes. But still, these alternatives contain nicotine and other substances such as propylene glycol. The potential long-term effects of these components on the ovarian reserve are currently unknown. Nicotine exerts its effects by binding to nicotinic acetylcholine receptors (nAChRs) and we have previously shown that specific nAChRs are expressed in small follicles in the mouse ovary.

Aims: The aim of this study is to analyse effects of nicotine on small follicle maintenance and activation. Methods Ovaries from 4-day old mice (replete with primordial to secondary stages) and preantral follicles (>secondary stage) from 16-day old mice were cultured for 7 days and 3 days respectively using different concentrations of nicotine 0 (control), 5 (low), 15 (medium) or 45 (high) ng/ml -- in accordance with published serum levels from e-cigarette users. Measurements of oocyte and follicle size were determined from microscopic images.

Results: Follicles from cultured, neonatal ovaries exposed to each dose of nicotine (low, medium, high) were slightly reduced in size relative to controls. Further analysis showed this effect of size was detectable in both oocytes and granulosa cells. When follicles were stratified by stage, nicotine had no observable effect on primordial follicles, but caused a slight, significant reduction in size of transitional, primary and secondary follicles. By comparison, nicotine had no detectable effect on the growth of larger, preantral follicles.

Conclusion: This study raises the possibility that nicotine can cause subtle effects on small follicles, which is consistent with previous receptor localisation; however, the long-term consequence on ovarian function and fertility are unknown.

P219 Myometrial function and viability following controlled-rate cryopreservation of ovine uteri

Jaffer Sabrina; Elmes Matthew; Hernandez-Medrano Juan

University of Nottingham

Uterine cryopreservation maintains viability and function, and represents a long-term storage option for transplantation^{1,2}. However, it was reported to decreased myometrial contractile function compared to fresh controls. This study aims to investigate the effect of slow-freezing cryopreservation (SF) on uterine contractility in response to increasing oxytocin (OT) concentrations in the ovine uterus. Uterine horns were perfused (0.5ml/min) with cold heparinised-solution followed by CPA (0.1M sucrose, 1.5M Me₂SO, 10% FCS in Leibovitz-L15) for 60 (SF60, n=4) or 75min (SF75, n=4) and cryopreserved using a controlled-rate freezer (Planer Ltd). Fresh uterine horns served as controls (CT; n=4). Non-caruncular tissue strips (0.5cm x 1cm) were dissected, mounted and kept in 25ml-organ baths containing KH buffer (37°C; gassed with 95% O₂ / 5% CO₂). Baseline tension (g), contraction frequency (peaks/min) and contraction force (AUC; g/s) were recorded before and after OT cumulative doses (0-9.5ng/ml)³. Ratio of vascular and myometrial layers (H&E staining) and connexin-43 staining (Cx43; Abcam) were evaluated for morphology and tissue viability. Contractility data (LabChart v6) was analysed using ANOVA for repeated measures, while morphology and Cx43 compared using Kruskal-Wallis tests, respectively. Spontaneous ex vivo myometrial contractions were evident in CT but not in SF (P<0.05). OT increased frequency (P<0.05) and force (P<0.05) in CT with no response elicited in SFs. Conversely, OT increased basal tension in all tissues (P<0.05) but to a larger extent in CT (P<0.05). Ratio of vascular to myometrium layers increased (P=0.1) while Cx43 staining decreased (P=0.02) in SF compared to CT uteri. In conclusion, cryopreservation decreases uterine function. This may result from increased tissue damage (decrease Cx43) caused by cryopreservation or perfusion (increased vascular layer). New strategies to reduced damage and improve cryopreservation protocols need to be developed.

1. Brännström M, et al. 2012. Uterus transplantation: animal research and human possibilities. *Fertility and Sterility*. 2012; 97:1269-1276. 2. Dittrich R, et al. 2010. Uterus cryopreservation in the sheep: one step closer to uterus transplantation. *In Vivo*. 24:629-634. 3. Elmes M, et al. 2015. Maternal age effects on myometrial expression of contractile proteins, uterine gene expression, and contractile activity during labor in the rat. *Physiol. Rep*. 3:1-23.

P220 Prevalence of eating disorders amongst pregnant women attending antenatal care at a tertiary teaching hospital in north-west Nigeria: outcome with the use of a screening tool

Gazali Sakina; [Adesiyun Adebiyi](#)

Ahmadu Bello University Teaching Hospital

Background: In the developed countries, there has been an upsurge of eating disorders necessitating conscious effort to diagnosing it in pregnancy because of its impact on the mother and the developing fetus (1.,2.,3).

Objective: To determine the prevalence of eating disorders in pregnant women attending the antenatal clinic of Ahmadu Bello University Teaching Hospital , Zaria.

Method: This was a cross-sectional study that was conducted to determine the prevalence of eating disorders in pregnant women using the SCOFF questionnaire (4). Respondents were recruited from the antenatal clinic of Ahmadu Bello University Teaching Hospital, Zaria, Nigeria between August-October 2018.

Results: The prevalence of eating disorders in pregnancy was 25.2%. Thirty two (84.2%) of the respondents with eating disorders felt they had lost control over how much they eat accounting for the highest response. Also, 78.9% (n=30) of the respondents said food dominates their life. These were based on the SCOFF questions. The ages of the respondents with eating disorders in pregnancy ranged from 16 to 39 years with a mean age of 28.2 years (SD: ± 5 years). Over 70% of the respondents with eating disorders in pregnancy were aged 29 years and below. Majority of the participants belonged to the Hausa ethnic group accounting for 71%, 97% of them were married, 60% had a lower educational status and 60.5%% were unemployed. Sixty percent of the respondents were aware of eating disorders in pregnancy. The data was analyzed using SPSS 20 version.

Conclusion: There is a high prevalence of eating disorders in pregnancy in this environment and the antenatal period is an opportunity to identify such patient so as to ensure continuity of care and effective referral to the mental health physician.

Keywords: Eating disorders, pregnancy, prevalence.

1. Mitchell JE, Seim HC, Glotter D, Soll EA, Pyle RI. A retrospective study of pregnancy in bulimia nervosa. *Int J Eating Dis* 1991; 10:209. 2. Brinch M, Isager T, Telstrup K. Anorexia nervosa and motherhood: reproduction pattern and mothering behaviour of 50 women. *Acta Psychiatr Scand* 1988; 77:611. 3. Kins MB. Eating disorders in a general practice population. Prevalence, characteristics and follow-up at 12 to 18 months. *Psychiatr Med* 1989; 14:1. 4. Morgan JF, Reid F, Lacey JH. The SCOFF questionnaire: a new screening tool for eating disorder. *West J Med* 2000; 172: 164-165.

P221 Microsurgical Testicular Sperm Extraction (micro-TESE) for azoospermia at a large tertiary referral centre: demographics and histopathological results

Kwok Abigail¹; Baird Andrew¹; Drakeley Andrew²; Lucky Marc¹

¹Aintree University Hospital NHS Foundation Trust; ²Liverpool Women's NHS Foundation Trust

Background/objectives: Micro-TESE for azoospermia is the gold standard treatment for providing men with the highest chance of obtaining sperm for assisted conception. We analysed histopathological results to investigate underlying associations or causative factors in men with non-obstructive azoospermia.

Methods: Results of azoospermic men undergoing micro-TESE at our centre between May 2015 and August 2019 were obtained. Patient demographics and histopathological results were analysed.

Results: 102 men had micro-TESE. Mean age was 34 years (range 17-50). Mean BMI was 27 (range 18-48). 86% were non-smokers, 14% ex-smokers and 14% current smokers.

Demographics included: 13% of men had previous urological diagnoses: orchidectomy (n=6), orchidopexy (n=4), orchitis (n=2), varicocele embolization (n=1). Comorbidities included: asthma (12%), previous cancer (11% of which 55% were testicular cancer), gastro-oesophageal reflux (6%), Klinefelter syndrome (6%), cardiovascular disease (5%), neurological disease (4%), liver disease (3%), infectious disease (3%), trauma (4%), diabetes (2%), inflammatory bowel disease (IBD) (1.2%) and renal transplant (1%).

The average Johnsen score was 3.1 (range 1-9). 59.8% of histopathology showed Sertoli cell only syndrome (SCOS). 9.7% of patients had acellular, empty tubules. 7.8% had fibrous obliteration of the lumen from sloughing of the epithelium. One patient had evidence of testicular cancer, however this was suspected prior to micro-TESE. All other patients had germ cells present, 20.6% contained spermatozoa. Nine patients had one testis sampled (due to previous orchidectomy, significant atrophy of contralateral testis, large varicocele and due to previous surgical scarring). Of patients with bilateral samples 87.5% had the same histology from both sides.

Conclusions: The majority of men undergoing micro-TESE for azoospermia have SCOS. There is variation of past medical history (PMH) with significant proportion of men having previous urological problems/surgery. Other PMH relate to treatment of previous cancer, asthma, and IBD involving chemotherapy/radiotherapy/steroids, which could potentially impact sperm production and warrants further research.

P223 Sertoli only cell syndrome in non-obstructive azoospermic men: what can we do to increase sperm yield from micro-TESE?

Kwok Abigail¹; Baird Andrew¹; Drakeley Andrew²; Lucky Marc¹

¹Aintree University Hospital NHS Foundation Trust; ²Liverpool Women's NHS Foundation Trust

Background/Objectives: Sertoli Cell Only Syndrome (SCOS) occurs when seminiferous tubules are lined by sertoli cells only with absence of germ cells. 10.8-44% cases of azoospermia are caused by SCOS¹⁻³. We analysed demographics of this group to identify possible links with the aim of increasing male fertility in this subset of patients.

Methods: Retrospective analysis of patients undergoing microsurgical testicular sperm extraction (micro-TESE) in a single tertiary referral centre from March 2015 to August 2019 was undertaken. Patients had histopathology and demographic factors evaluated.

Results: 102 micro-TESEs were performed. SCOS was identified in 61 (59.8%). This was bilateral in 80% of cases (4 cases only sampled unilaterally due to previous orchidectomies for cancer).

Mean BMI was 26.8 (range 17.9-48). Mean age was 34.1 years (range 26-45). Past medical history included: reflux, (2%) asthma (7%), inflammatory bowel disease (3%), previous cancer (5%), previous urological problems/surgery (orchidectomy (6.5%), orchidopexy (5%), embolization of varicocele (1.6%)), cardiovascular disease (2%), neurological conditions (3%), liver disease (2%), diabetes (2%), infectious disease (5%), Klinefelter syndrome (3%) and trauma (2%). Mean Johnsen score 2.13 (range 1.03-4.25).

Of the SCOS group, 6 men had successful sperm extraction (10%). This implies that they had areas if tubules not affected by SCOS. They had mean age of 32.7 years and BMI of 26.8. All were non-smokers. All but one had PMH including: chronic hepatitis (16.7%), previous Ewing sarcoma (16.7%), asthma (16.7%), previous testicular cancer and orchidectomy (16.7%) and spina bifida (16.7%). Average Johnsen score was 2.24.

Conclusion: SCOS is a common cause of azoospermia associated with a low yield of successful sperm extraction (10%). We did not identify any modifiable factors to improve success. Further research into this area with larger patient populations may yield clinically significant results.

1. Sasagawa I, Yazawa H, Suzuki Y, Tateno T, Ichihara O, Kobayashi T et al. Re-evaluation of testicular biopsies of males with non-obstructive azoospermia in assisted reproductive technology. *Archives of Andrology*. 2001;46:79-83.
2. Mitchell C, Robin G, Boitrelle F, Massart P, Marchetti C, Marcelli F et al. Correlation between testicular sperm extraction outcomes and clinical, endocrine and testicular histology parameters in 120 azoospermic men with normal serum FSH levels. *International Journal of Andrology*. 2001;34:299-305.
3. Abdullah L, Bondagii N. Histopathological patterns of testicular biopsy in male infertility: A retrospective study from a tertiary care center in the western part of Saudi Arabia. *Urology Annals*. 2001;3(1):19-23.

P224 Evaluation of patient information leaflets provided in fertility centres by the Flesch and Flesch-Kincaid method

Aravind Niveda¹; Mctavish Alison²; Maheshwari Abha³

¹Robert Gordon College; ²University of Aberdeen; ³NHS Grampian

Background: Health literacy is a priority to the NHS due to its impact on patients' healthcare and outcomes. IVF is complex with a lot of unfiltered information available on the internet. Clinics must give appropriate and adequate information in a timely way to ensure patients can give informed consent and to meet the requirements of the HFEA. Patient information leaflets (PILs) are a key source of medical information. There is a concern that the information provided in PILs, may exceed patient comprehension, thus negating their beneficial effect, and patient's understanding of the treatments.

Objectives: This study aimed to analyse Patient Information Leaflets at the Fertility Centre in terms of readability using Flesch Reading Ease score (FRES) & Flesch Kincaid Grade (FKG) and compare with patient satisfaction.

Methodology: Nine most commonly used leaflets were chosen. Readability statistics on these leaflets, collected from analysing the leaflets, was compared with national literacy statistics (FRES & FKG). A patient survey was conducted between March and May 2019 using a short anonymised questionnaire. Results: Thirty-six responses were received from the initial 49 questionnaires handed out. The PILs had an average FRE score of 50.7 and an average FKG Level of 11.0 suggesting that the PILs would be challenging reads for 43.4% of the working population in the UK; however, the leaflets achieved a patient satisfaction rate of 92%.

Conclusion: Although our PILs were well laid out and easy to read, the majority would have exceeded patient comprehension according to FRES & FKG. The current advice for provision of patient information does not highlight the importance of a recommended reading level when designing a PIL, potentially excluding a wide group from the benefits of a PIL. In future, all leaflets will, be modified taking in consideration the readability score in addition to other requirements.

P225 Evaluation of return rates of female oncofertility patients and a comparison with male return rates

Davies Maddison¹; Shehadeh Zachariah²; D'Angelo Arianna²; Zaki Mona²; Jose Grace²; Kalra Gurpreet²

¹Cardiff University Medical School; ²Wales Fertility Institute

Objective: To analyse the return rates of female oncofertility patients, compare these to male return rates at the same facility, and analyse reasons for non return.

Methods: Data was collated from databases across 2 facilities dating from 2007 to present. Patients selected were females cryopreserving oocytes or embryos for fertility preservation in the event of cancer. Outcomes were categorised as either samples discarded, patients deceased, storage extended, no update at present, or frozen embryo transfer completed.

Results: 67 patients were included in the study which found a 22% return rate of patients thawing their samples, and 5 live births from the patients proceeding to FET. 7 patients had their samples discarded due to consent expiry, 6 patients were deceased, 3 patients extended their storage and there was no update for 33 patients. In the parallel study completed at the same facility for male oncofertility (1. Powis 2019) the return rate for samples was 2.4%, from 2014 to present, 14 of 585 patients. 38 male participants discarded their samples due to consent expiry.

Conclusions: NICE guidelines suggest storage is offered for a minimum of 10 years, costing up to 350 pounds per annum for oocyte preservation and 450 for sperm. This data suggests this timeline should be evaluated in view of cost effectiveness, with only 22% of patients returning to retrieve their samples from storage and a considerable proportion of samples ending up discarded simply due to consent expiry. We propose a check up part way through storage time to re-establish patients interest in fertility preservation treatment, so samples which are no longer necessary can be discarded of sooner and money saved.

Powis L. The chronology of sperm sample storage and use in patients attending a national fertility preservation centre. Human Reproduction Abstracts: ESHRE; 2019.

P227 The effect of unincorporated cells on blastocyst grade and treatment outcome

Neville Konstancja; Smale Daniela; Gunn Joel; Price Anthony; Ingamells Sue

Wessex Fertility

Background: Simplified blastocyst grading (SBG) was adopted at the clinic in 2018. SBG provides a simple alternative to conventional grading schemes, aiming to reduce assessment time and practitioner subjectivity while predicting implantation potential. SBG categorises blastocysts into grades A-D depending on inner cell mass (ICM) and trophectoderm (TE) morphology and the presence of unincorporated cells; the presence of one unincorporated cell warrants grade C, whereas two or more unincorporated cells result in grade D classification. Although grade A-D criteria were strictly applied based on ICM and TE quality, the categorisation of blastocysts into C-D grades due to the presence of excluded cells was relatively lenient. The aim of this study was to evaluate the effect of unincorporated cells on blastocyst grade and treatment outcome.

Methods: Descendent frozen embryo transfer data was collected retrospectively from fresh IVF/ICSI cycles performed between January 2018 and June 2019. Expanded blastocysts were regraded using strict SBG criteria. Grade changes were noted and the differences in cycle outcome between the current grade (CG) and SBG were analysed using the Mann-Whitney test.

Results: The regraded blastocyst data showed that 60% of the embryos received a different grade with SBG, where 41% of embryos were reclassified as grade D. There were no statistically significant differences in implantation and clinical pregnancy rates between CG and SBG grade A (73.5% vs 81.3%, $p = 0.79$ and 62.9% vs 73.7%, $p = 0.76$), and grade C embryos (38.7% vs 53.8%, $p = 0.15$ and 30% vs 42.9%, $p > 0.99$). SBG grade D embryos resulted in 54.1% implantation rate and 42.9% clinical pregnancy rate.

Conclusions: Strict adherence to SBG showed a substantial difference in grade distribution. The presence of unincorporated cells did not have a significant impact on cycle outcome. Introduction of a minus subgrade categories may prove beneficial in reducing embryo wastage.

P228 Obstetric outcomes in women with severe ovarian hyperstimulation syndrome following assisted reproduction: a case-control study

Oatham Rosie¹; Cheung Candice²; Kasraie Jason²; Sizer Andrew²

¹Keele University School of Medicine; ²Shrewsbury and Telford Hospital NHS Trust

Objectives: to assess the obstetric outcomes in women diagnosed with severe ovarian hyperstimulation syndrome (OHSS) following in-vitro fertilisation (IVF) or intra-cytoplasmic sperm injection (ICSI). Background: OHSS is an iatrogenic complication of assisted reproductive technology (ART). Medical therapy in OHSS aims to reduce maternal morbidity, such that obstetric outcomes often become a secondary focus. Various factors have been implicated in the pathophysiology of OHSS, but there remains a paucity of data regarding the impact of OHSS on developing pregnancies.

Methods: A retrospective case-control study comparing obstetric outcomes in 18 patients hospitalised with severe OHSS following ART, to a group of patients with matched characteristics in whom OHSS did not occur, over a 10-year period.

Outcome measures: Miscarriage rate, pre-term delivery rate, birthweight, foetal anomalies, obstetric complications.

Results: Five patients had a "freeze all" cycle. Thirteen patients underwent embryo transfer in both groups, and all achieved a biochemical pregnancy. Eleven (84.6%) patients achieved a clinical pregnancy following OHSS with a livebirth rate of 69.2%. Patients hospitalised with OHSS were compared to 13 patients with similar demographics who achieved a biochemical pregnancy. Miscarriage rate was higher in pregnancies complicated by severe OHSS (30.77% vs 7.69%). We compared the outcomes of 4 singletons and 5 twin births in the OHSS group, to 7 singletons and 5 twin births in the non-OHSS group. Multiple pregnancy rates were similar between the groups (55.56% vs 41.67%). Patients who developed severe OHSS had a greater incidence of pre-term delivery (44.4% vs 8.3%). The mean birthweight recorded was lower in the OHSS group (2,794 ± 764g vs 2963 ± 640g). No foetal abnormalities were noted. There were no recorded cases of gestational diabetes, pregnancy-induced hypertension (PIH) or placental abruption in either group.

Conclusions: Higher rates of miscarriage and preterm delivery are found in the severe OHSS group.

P229 Injudicious Clomiphene Citrate (CC) therapy in the secondary care setting

Bamford Thomas; Kingsland Charles

Lancashire Teaching Hospital Trusts

Introduction: NICE guidance clearly states, 'do not offer ovarian stimulation agents to women with unexplained infertility'. This view is supported by a Cochrane review that demonstrated no clinical benefit [1]. We hypothesised that in practice empathic clinicians may be influenced to prescribe CC to mitigate a patient's diagnosis, particularly in the face of IVF costs. However, it can cause unfavourable changes in cervical mucus, may reduce endometrial thickness and cause multifetal gestation or side effects [2].

Method: A mixed methods retrospective audit of case notes in a 6-month period between June and November 2018 (n=234). Phone calls to these patients were used to elicit any CC side effects and determine pregnancy rates. We also conducted a national survey among fertility specialists to explore compliance with guidance.

Results: Unexplained infertility was diagnosed in 76 patients (32%). Other diagnoses included: male factor infertility (13%), diminished ovarian reserve (11%), WHO classification I (0.8%), II (26%), III (2%), and others (7.7%). 21 (55%) of the patients with unexplained infertility were prescribed CC and 38 (71%) referred for IVF (13 of these had a trial of CC therapy prior). 142/234 patients agreed to be interviewed. The three most common side effect was hot flushes (12.6%), abdominal discomfort (7%), nausea and vomiting (3%), and one patient had OHSS. 4/142 (2.8%) patients interviewed with unexplained infertility became pregnant using only CC vs. 40/142 (28%) of patients referred for IVF. A

survey of clinicians revealed 50% (10/20) consultants never prescribe CC in unexplained infertility, with 30% rarely (6/20) and 20% occasionally (4/20).

Conclusions: This project illustrates significant non-compliance with guidelines. We used qualitative techniques to suggest reasons for this and make recommendations for changes in practice. Although prone to information bias, patient interviews highlight that CC prescriptions aren't always without consequence.

1. Hughes E, Brown J, Collins JJ, Vanderkerchove P. Clomiphene citrate for unexplained subfertility in women. Cochrane Database of Systematic Reviews 2010, Issue 1. Art. No: CD000057. DOI: 10.1002/14651858.CD000057.pub2 2. Gadalla, M. A., Huang, S., Wang, R. et al. Effect of clomiphene citrate on endometrial thickness, ovulation, pregnancy and live birth in anovulatory women: systematic review and meta-analysis. Ultrasound Obstet Gynecol. 2018; 51:64-76.

P230 USE of intracytoplasmic morphologically selected sperm injection (IMSI) reduces risk of miscarriage and increases embryo utilisation in patients with high sperm DNA damage

Sowry Blair¹; Odia Rabi¹; Lewis Sheena²; Serhal Paul¹; Mowla Shahriar³; Khanjani Shirin¹; Ben-Nagi Jara¹

¹The Centre for Reproductive and Genetic Health; ²Examen; ³Imperial College London

Introduction: Sperm DNA damage is known to have a negative impact on reproductive outcomes with links to poor embryo development, increased risk of miscarriage and lower live birth rates. Various interventions have been proposed to improve reproductive outcomes in patients with high sperm damage. One such intervention is intracytoplasmic morphologically selected sperm injection (IMSI), where sperm are selected under high magnification (x6000) for an absence of vacuoles prior to injection. Vacuolated sperm has been correlated with higher sperm damage, although the evidence for IMSI resulting in an improved reproductive outcome for patients with increased DNA damage has been inconclusive.

Methods: A retrospective analysis was carried out on couples with high sperm DNA fragmentation using the Comet assay clinical thresholds who had embarked on conventional IVF (n=31), ICSI (n=56) or IMSI (n=86) treatment. Fertilisation, blastocyst development, embryo utilisation, clinical pregnancy and miscarriage rates were calculated for the three treatment groups. Statistical significance between the groups was determined using chi-squared analysis ($p < 0.05$).

Results: There was a significant increase in embryo utilisation rate with both IMSI and ICSI treatment compared with conventional IVF ($p < 0.05$). The risk of miscarriage was significantly reduced in both the IMSI and ICSI groups compared with conventional IVF treatment ($p < 0.05$). No statistical differences were observed between the three treatment groups in relation to fertilisation rate, blastocyst development or clinical pregnancy rate. The mean female patient age was not statistically different between the three study groups.

Conclusion: Although evidence for the use of IMSI as a clinical intervention in patients with high sperm DNA damage is limited, our data suggests that the use of both ICSI and IMSI results in a lower risk of miscarriage. Moreover, the use of IMSI or ICSI treatment for this group, also results in a higher rate of embryo utilisation compared to IVF.

P231 Which is the best AMH or AFC in predicting ovarian response in IVF for patients with discordant AMH AFC results?

Abdulah Alaa¹; Raine-Fenning Nick²; Polanski Lucas²

¹University Hospital of North Durham; ²Nottingham University Research and Treatment Unit in Reproduction and Infertility

The current evidence suggests that AMH & AFC are the best markers of ovarian reserve. Both are highly positively correlated to each other; however, this may not be usually the case. Some patients have AMH & AFC disagreement. We are trying to explore which is the best AFC or AMH in prediction of ovarian response in patients with discordant AMH AFC results. We plotted the values of AMH against AFC values in 30 studies using on-line search, and AMH was correlated with AFC in a regression equation of ($AFC = 1.65 AMH + 6.05$) (AMH measured in ng/ml) and a regression equation of ($AFC = 0.22 AMH + 6.05$) (AMH measured in pmol/l). A total of 250 patients were identified with AMH &

AFC results in our retrospective study in NURTURE. Linear regression showed a correlation coefficient of 0.57 and ($P < 0.001$), with a regression equation of $AFC = 0.488 AMH + 9.55$. 60 cases with discordant Amh AFC results were identified that then were divided into four groups: group IA (low AFC normal AMH), group IB (high AMH normal AFC), group IIA (high AFC normal AMH) and group IIB (low AMH normal AFC). Group IA had a significantly lower percentage of low response (61%) than group IIB (9%) with lower number of oocytes retrieved. The percentage of high response was higher (50%) in group IB compared to group IIA (41%). Both AMH & AFC were correlated with the number of oocytes retrieved ($R = 0.62$ & 0.50 , respectively). Both AFC & AMH were significant predictors of the oocytes retrieved according to linear regression results ($P < 0.001$). Logistic regression & ROC curve analysis confirmed that AMH was more significant predictor of the poor response with $AUC = 0.948$ compared to AFC which yielded an AUC of (0.78). AMH was also slightly superior over the AFC in the excessive response prediction (> 14 oocytes retrieved) (AMH $AUC=0.76$) vs. (AFC $AUC= 0.72$). Both AMH & AFC were not significant predictors of the clinical pregnancy ($AUC = 0.56$ and 0.52 , respectively). To conclude, AMH had better prediction of the number of oocytes retrieved than AFC in those patients.

P232 A re-evaluation of NICE guidance for donor insemination: evidence from 8.922 consecutive treatments from a single centre

Bodri Daniel; Linara-Demakakou Elena; Wang Jinjun; Lamanna Giuseppina; Arian-Schad Mimi; Nair Shailaja; Macklon Nick; Ahuja Kamal

London Women's Clinic

Aim: Current UK clinical guidelines (NICE, 2017) for same-sex couples recommend up to 12 intracervical/intrauterine insemination treatments, whereas for heterosexual couples IUI with partner sperm is not advised as a first-line treatment. This non evidence-based national policy has drawn fierce criticism from practitioners because a potentially efficient, less invasive and cheaper treatment is denied to patients. To investigate the appropriate number of cycles to offer, we conducted a retrospective, single-centre cohort study in a private, HFEA-regulated centre.

Methods: Between 2004-2016, 3.333 consecutive women (45% single, 43% in a same-sex and 12% in a heterosexual relationship) underwent 8.922 cycles. Of these 58% were natural and 42% clomiphene/gonadotropin stimulated.

Results: Seven-hundred ninety-five live births occurred (8.9% per cycle delivery rate) including 24 (3%) twins. Respective age-specific crude and expected cumulative live birth rates were 29, 23, 21, 12% and 66, 49, 54, 28% in under 35, 35-37, 38-39- and 40-42-year-old patients. A clear plateau was reached after 6 and 3 attempts in the <40 and 40-42-year age groups, respectively. There were no significant differences in cumulative success rates between lesbian patients and single women or heterosexual patients. In a multivariate analysis, age (aOR: 0.92 95%CI:0.90-0.93), previous IUI-D live birth (aOR: 2.15 95%CI:1.69-2.73) and ovarian stimulation (aOR: 1.27 95%CI:1.09-1.48) were significantly associated with higher success rates.

Discussion: Acceptable cumulative live births rates rising over 6 cycles were achieved in a large donor insemination programme involving mainly lesbian couples and single women. Mild ovarian stimulation and previous IUI-D success were associated with increased chance of pregnancy. In contrast to current UK clinical guidelines, up to 6 treatment cycles (but not 12) are recommended in women <40 years of age and 3 attempts seem to be reasonable up to 42 years of age, irrespective whether donor or partner sperm is used.

P233 A national survey of fertility preservation in clinical practice across the United Kingdom in paediatric oncology specialist centres amongst under 18 year olds with cancer

Newton Hannah¹; Friend Amanda¹; Hayden Catherine²; Skinner Roderick³; Picton Helen¹; Glaser Adam⁴

¹University of Leeds; ²Leeds Teaching Hospitals Trust; ³Great North Children's Hospital; ⁴University of Leeds and Leeds Children's Hospital, Leeds Teaching Hospitals Trust

Background: There is an impression of variation in access to, and funding of, fertility preservation options for children with cancer across the United Kingdom (UK). We aimed to establish current provisions and practices.

Methods: UK children's cancer services are delivered by a network of 20 specialist centres. Each was invited to participate in an online survey of referral rates, service availability and funding arrangements.

Results: were analysed in geographical clusters for anonymity. Results 90% (18/20) of centres responded, covering 92% of new referrals per year across UK. 100% of centres had referred patients for fertility preservation over the last 12 months. Variation in services provided and funding arrangements were reported: Males, post-pubertal (sperm): 17/18 centres had referred in the last year. No geographical variation in funding source was identified: 88% National Health Service (NHS)/Health Board, 12% unsure. Inconsistency in length of funding was reported (range: <5years to indefinite). Males, pre-pubertal (testicular tissue): 15/18 centres have referred. Funding sources vary: 50% charity, 11% NHS/Health Board, 11% research, 6% cancer network, 5% case-by-case public funding, 17% unsure. Inconsistency in funding duration exists (range: 10years to indefinite). Females, post-pubertal (eggs / embryos): 6/18 centres have referred. Funding sources vary: 50% NHS/Health Board, 11% charity, 11% case-by-case public funding, 11% other, 17% unsure. The duration of funding is inconsistent (range: <5years to indefinite). Females, pre-pubertal (ovarian tissue): 18/18 centres have referred. Funding sources vary: 50% charity, 17% NHS/Health Board, 11% research, 6% cancer network, 5% case-by-case public funding, 11% unsure. The duration of funding is inconsistent (range: 10years to indefinite).

Conclusions: Other than for sperm storage in post-pubertal males, significant variation in referral patterns and funding sources existed across the UK for pre-pubertal males and all females aged under 18 years. Urgent clinical and commissioning guidance is needed to ensure equitable access.

P236 Developing a core definition set for future infertility research and reporting: an international consensus development study

Beebeejaun Yusuf¹; Duffy James²

¹King's Fertility; ²Cochrane Gynaecology and Fertility Group, University of Auckland, New Zealand

Infertility trials are unique. Their outcomes reflect maternal, paternal, embryonic and fetal parameters which are often managed by different specialists guided by different guidelines. With an increased amount of research and international collaborations within the field of infertility, the results of randomised controlled trials as well as clinical outcome reporting often differ since there is currently no standardised protocol of reporting outcomes within infertility trials and research. Current standards such as the Consolidated Standards of Reporting Trials (CONSORT) guidelines, have not been updated to reflect these unique issues of infertility trials making it difficult for academics and clinicians to compare results and adopt recommendations. We therefore aim to standardise infertility research by developing a core definition set which will be used to define future randomised controlled trials and systematic reviews evaluating potential treatments for infertility. A core definition set for infertility research was agreed upon by an internal consortium. Current definitions set and in practice were researched and compared through a systematic review process. This included terms defined by Improving the Reporting of Clinical Trials of Infertility Treatments (IMPRINT), the International Committee Monitoring Assisted Reproductive Technologies (ICMART), Cochrane systematic reviews as well as definition development initiatives including National and international clinical practice guidelines. We used consensus development methods including the representativeness of the participant sample, Delphi survey attrition, and an arbitrary consensus threshold in order to set the parameters of reporting. Our future work include standardising the identified definitions through a consensus meetings thereby developing a core definition set for future randomised controlled trials and systematic reviews evaluating potential treatments for infertility. By incorporating these internationally agreed definition set within future infertility research, we aim to improve the impact of research and facilitate laboratory and clinical research into clinical practice with the aim of enhancing the care people with infertility problems receive.

Dapuzzo L, Seitz FE, Dodson WC, Stetter C, Kunselman AR, and Legro RS. Incomplete and inconsistent reporting of maternal and fetal outcomes in infertility treatment trials. *Fertility and Sterility* 2011; 95: 2527-2530. Duffy JMN, Bhattacharya S, Curtis C, Evers JLH, Farquharson RG, Franik S, Khalaf Y, Legro RS, Lensen S, Mol BW, et al. A protocol developing, disseminating and implementing a core outcome set for infertility. *Human Reproduction Open* 2018; 2018.

P239 Does the Endometrial Receptivity Array test improve pregnancy rates in patients with recurrent implantation failure?

Chinn Victoria; Newberry Megan; Joy Jolly

CARE Manchester

Introduction: Recurrent implantation failure (RIF) is seen in up to 10% of women with repeated IVF failure. A displaced window of implantation (WOI) is one cause of RIF. Identification of a personalised receptive WOI by the expression of various genes in the endometrium by the endometrial receptivity array (ERA) test has been a highly debated topic.

Aim: To determine whether the ERA test and adjusting the embryo transfer (ET) day, according to the shift in WOI improves the pregnancy rates for RIF patients.

Method: A retrospective review of 35 patients who had the ERA test between October 2017 - June 2019 was performed. Collectively they had undergone 147 ETs. The ET time and progesterone support was timed in correlation with the number of hours recommended by the ERA result.

Results: Of the 35, 23 patients were found to be receptive after the routine 5 days of progesterone support, 12 patients were non receptive -- pre receptive (n=5), early receptive (n=2), late receptive (n=4), post receptive (n=1). 28 patients went on to have ETs, 7 of which had pre implantation genetically tested embryos replaced. Of all the ETs performed, 15 patients went on to have negative pregnancy tests, 10 had a positive test result of which 5 had a live birth outcome. 3 patients are still awaiting their pregnancy test results. Out of the 10 positive pregnancy tests, 8 had received a receptive result and the remaining 2 had received a pre receptive ERA result.

Conclusion: Providing an ERA test for patients diagnosed with RIF can personalise their ET. However, it is unclear whether this significantly improves the pregnancy rates. More research is required and further studies with larger cohorts would be necessary to find out the statistical significance of the ERA test.

P240 Patients with AMH AFC discordance in IVF, which is the best to predict ovarian response AMH or AFC in patient with Discordant AMH AFC results in IVF

Abdulah Alaa¹; Raine- Fenning Nick²; Polanski Lucasz²

¹University Hospital of North Durham; ²Nottingham University Research and Treatment Unit in Reproduction and Infertility

Ovarian reserve defined as the pool of follicles residing in the ovary at any particular time both qualitatively and quantitatively. The current evidence suggests that AMH & AFC are the significant markers of ovarian reserve.. Both are highly positively correlated to each other; however, this may not be usually the case. Some patients have AMH & AFC disagreement. It is challenging to decide which test is reliable for those patients. Since both AMH & AFC have the same degree of predictability of the oocyte yield, we will test the hypothesis that states there should be one predictive marker either AMH or AFC for patients with discordant AMH & AFC values. A retrospective study was performed in which AMH & AFC values of 250 patients were classified according to percentile rank. 60 cases with discordant results (according to the percentiles of both AMH & AFC values) were identified that then were divided into four groups: group IA (low AFC normal AMH), group IB (high AMH normal AFC), group IIA (high AFC normal AMH) and group IIB (low AMH normal AFC). Group IA had a significantly lower percentage of low response (61%) than group IIB (9%) with lower number of oocytes retrieved. Similarly, the percentage of high response was higher but not statistically more significant (50%) in group IB compared to group IIA (41%). Both AMH & AFC were correlated with the number of oocytes retrieved ($R=0.62$ & 0.50 , respectively). Both AFC & AMH were significant predictors of the oocytes retrieved according to linear regression results ($P < 0.001$). However, logistic regression & ROC curve analysis confirmed that AMH was more significant predictor of the poor response with $AUC=0.948$ compared to AFC which yielded an AUC of (0.78) . AMH was also slightly superior over the AFC in the excessive response prediction (> 14 oocytes retrieved) (AMH $AUC=0.76$) vs. (AFC $AUC= 0.72$). Both AMH & AFC were not significant predictors of the clinical pregnancy ($AUC=0.56$ and 0.52 , respectively). In conclusion, AMH had better predictive association with the number of oocytes retrieved than the AFC. It may be a more reliable test than the AFC if there was a discrepancy in the AMH & AFC results.

P241 Embryo quality, morphokinetics and clinical pregnancy outcomes: a comparison between three different gamete/embryo single-step culture media

Williams Hannah¹; Carroll Michael²; Drew Ellen¹

¹NHS Tayside; ²Manchester Metropolitan University

Background: With the introduction of continuous time-lapse monitoring, single-step culture media has enabled uninterrupted embryo culture. There are several commercially available CE-marked single-step culture media, however studies have not yet proven which promotes the best clinical outcomes. This study selected three market-leading single-step culture media to trial sequentially for a year. This study aimed to identify which had the highest fertilisation rates, embryo development rates and clinical pregnancy rates, and to explore effects on embryo morphokinetics.

Methods: Three commercially available CE-marked single-step culture media were selected for further study: G-TL™ (Vitrolife, Sweden), CSCM-C™ (Irvine Scientific, USA), and SAGE 1-step™ (CooperSurgical, Denmark). Results for IVF and ICSI embryos were analysed separately. Embryos cultured in EmbryoScope were annotated for morphokinetics, and key performance indicators (KPIs) such as fertilisation rates, embryo development and quality, and clinical pregnancy were analysed. Statistical analysis was performed using one-way ANOVA or Kruskal Wallis test. $p < 0.05$ was considered statistically significant.

Results: There were significant differences in the average time taken to reach pronuclear fading, 2 cell, 3 cell, 4 cell, 5 cell, 6 cell, 7 cell, morula, start of blastulation, blastocyst, expanded blastocyst and hatching blastocyst for embryos derived from IVF. There were significant differences in the average time taken to reach pronuclear fading, 2 cell, morula, time to blastocyst and expanded blastocyst for embryos derived from ICSI. Normal fertilisation rates, embryo quality and clinical pregnancy rates were not significantly different between the media for either IVF or ICSI cycles, however however there were significantly more 1PN embryos in the Vitrolife ICSI group.

Conclusion: Significant alterations in embryo morphokinetics are evident between the three media for embryos derived from IVF, however the effect is less prominent in ICSI-derived embryos. However, the morphokinetic variance did not translate into a clinical effect, as KPIs were not significantly altered.

P244 Defining the immune-epithelial IL-17 axis in the endometrium

Reidy Fiona¹; Giangrazi Federica²; Kavanagh Roisin²; Wingfield Mary¹; O'Farrelly Cliona²; Glover Louise³

¹Merrion Fertility Clinic; National Maternity Hospital; ²Trinity College Dublin; ³Merrion Fertility Clinic, Trinity College Dublin

Background: Successful embryo implantation requires complex interactions between the embryo and a receptive endometrium. Previous research in our group has found that dysregulation of the IL-17A pathway in the endometrium is linked to negative ART outcomes in cases of unexplained infertility. As of yet, the role IL-17 plays in successful embryo implantation, or indeed the endometrial cell populations involved in this process, has not been defined. Other work in our group has shown that IL-17 regulates expression of epithelial Antimicrobial peptides (AMP) in the female reproductive tract. Therefore, we hypothesise that IL17 may play a central role in modulating endometrial epithelial innate immune responses.

Methods: Patients were recruited when attending for assisted reproduction treatment ($n=5$). Endometrial pipelle biopsies were taken in the luteal phase of the menstrual cycle. 2 patients had a history of endometriosis, with the remaining 3 having no evidence of endometriosis. Localization of IL17 cytokine and IL17 receptor was investigated in endometrial biopsies using an immunohistochemical approach.

Results: In our cohort, immunostaining for IL-17A revealed localization to stromal immune cells, while the IL17 receptor IL17RA was shown to be largely epithelial cell-specific. These findings suggest that immune-cell derived IL-17 cytokine binds its receptor and signals through epithelial cells in the endometrium.

Conclusion: Increased IL-17 and innate immune factor (e.g. AMP) expression in the endometrium of patients who fail to achieve pregnancy may be reflective of functional changes in the endometrial innate immune milieu, and allow prediction of patients who are likely to have positive reproductive outcomes in ART.

P245 Natural vs medicated frozen embryo transfer: biochemical pregnancy rate

Gomes Catarina; Bird Sophie

Epsom and St Helier University Hospitals NHS Trust

Background: The most recent data from Human Fertilisation Embryology Authority (HFEA) shows that the number of in vitro fertilisation (IVF) cycles using frozen embryos increased by 11% since 2016 (1), and in 2017 the birth rate per embryo transferred was similar to cycles using fresh embryos (1). Critical for the success of a frozen embryo transfer (FET) program is synchronization between the endometrial development and the embryo.

Methods: Over the period November 2016 - July 2019 128 FET cycles using vitrified embryos, were initiated at our centre, and divided into two groups, the natural FET group and the medicated FET group.

Results: From 128 FET cycles initiated at our centre 43 were natural FET cycles and 85 were medicated FET cycles, with a cancellation rate of 14% in the natural cycle group and 8% in the medicated cycle group. Two did not reach the embryo transfer stage, one from each of the groups, due to failed thaw. Positive pregnancy test results (serum β -hCG) were similar for both groups (natural cycle 39%, medicated cycle 38%). There was a higher rate of biochemical pregnancies in the natural cycle group (16.7%) compared to the medicated cycle group (7.7%).

Conclusions: FET is an important part of the assisted reproduction techniques and certainly has brought many advantages to the field. The choice of endometrium preparation for a FET depends on several aspects, and there can be advantages to both natural FET and medicated cycles. In our study group both methods achieved similar positive pregnancy results but the natural cycle group had higher biochemical rate. Both natural and medicated should be offered to patients but it is not suitable for every patient. Following this study a decision was made to add progesterone support post LH surge to patients undergoing natural FET cycles (2).

(1) Hfea.gov.uk. (2019). Fertility trends explained | Human Fertilisation and Embryology Authority. [online] Available at: <https://www.hfea.gov.uk/about-us/publications/research-and-data/fertility-trends-explained/> [Accessed 5 Sep. 2019]

(2) Schwartz E, Bernard L, Ohl J, Bettahar K, Rongières C, Lichtblau I and Pirrello O. Luteal phase progesterone supplementation following induced natural cycle frozen embryo transfer: A retrospective cohort study. *Journal of Gynecology Obstetrics and Human Reproduction* 2019; 48(2):95-98. <https://doi.org/10.1016/j.jogoh.2018.10.011>