Reproduction Abstracts

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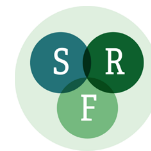

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Reproduction Abstracts

SRF Annual Conference 2016

11–13 July 2016, University of Winchester, UK

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Symposium 1: New technologies in reproductive science

S001

Can reproductive technologies prevent transmission of mitochondrial DNA disease?

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Mitochondrial DNA (mtDNA) mutations are maternally inherited and are associated with a broad range of debilitating and fatal diseases. Reproductive technologies designed to uncouple the inheritance of mtDNA from nuclear DNA may enable affected women to have a genetically related child with a greatly reduced risk of mtDNA disease. To this end, we have performed preclinical studies on pronuclear transplantation (PNT). Surprisingly, techniques used in proof of concept studies involving abnormally fertilized human zygotes were not well tolerated when applied to those that undergo normal fertilisation. We have therefore developed an alternative approach based on transplanting pronuclei shortly after completion of meiosis rather than shortly before the first mitotic division. The modified procedure, known as early PNT (ePNT), promotes efficient development to the blastocyst stage with no detectable effect on aneuploidy or gene expression. Following further optimisation, mtDNA carryover was reduced to $<2\%$ in the majority (79%) of PNT blastocysts. However, we found that 1/5 hESC lines derived from PNT blastocysts showed a marked increase in heteroplasmy despite relatively low (4%) starting levels. While the relevance of this to development in vivo is unclear, the finding underscores the importance of reducing mtDNA carryover to the lowest possible levels. We propose that the new ePNT procedure has the potential to give rise to normal pregnancies with a reduced risk of mtDNA disease, but may need further modification to eliminate disease in all cases.

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S002

Biallelic genome editing of human stem cells at scale

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The advent of site-specific nucleases and improved conditions for human iPSC culture now permits efficient engineering of human stem cells. CRISPR-Cas9 technology, in particular, provides a facile tool for the generation of a range of alleles in human stem cells with little risk of off-target damage. We established a high-throughput pipeline for the generation of homozygous knockout human iPSCs. We construct short arm targeting vectors and sgRNA expression plasmids in 96-well format. Following co-transfection of the targeting vector with Cas9 and sgRNA expression plasmids, we screen for clones where one allele is targeted by homologous recombination and the second allele is damaged by non-homologous end joining. Our method lends itself to high-throughput genotyping: biallelic events are identified by Sanger sequencing of the non-targeted allele. Bi-allelic knockout of genes is observed in 10–30% of the colonies screened. Our aim is to generate and distribute arrays of human iPS cell knockouts that will be coupled to focused phenotyping screens in cultured cells. Currently, we are developing a vector-free method using Cas9 ribonucleoprotein and single strand oligonucleotides for fluent generation of biallelic point mutations and revertants for disease modelling.

DOI: 10.1530/repabs.3.S002

S003

Monitoring dynamic changes of DNA methylation in single cells during development and disease

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DNA methylation is a broadly studied epigenetic modification that is essential for normal mammalian development. Over the years, numerous methodologies were developed trying to cope with the intrinsic challenge of reading the "second dimension" epigenetic code. The recent rapid expansion of sequencing technologies has made it possible to fully chart the methylation landscape of different cell types at single-base resolution. However, current methods provide only a static "snapshot" of DNA methylation, thus precluding the study of real-time methylation dynamics during cell fate changes. Therefore, a key challenge in the field is to generate tools that allow tracing real-time changes in DNA methylation. We have recently establish a Reporter of Genomic Methylation (RGM) that relies on a synthetic imprinted gene promoter driving a fluorescent protein. We showed that insertion of RGM proximal to promoter-associated CpG islands, or non-coding regulatory elements such as tissue-specific super-enhancer regions, allows faithful reporting on gain and loss of DNA methylation. Importantly, we demonstrated that RGM allows to trace real-time DNA methylation dynamics, at single-cell resolution, during cell fate changes. In placental mammals, differential DNA methylation at imprinting control regions (ICRs) regulates the parent-of-origin monoallelic expression of multiple imprinted genes in clusters. To study allele-specific methylation dynamics associated with ICRs during mouse development, we targeted RGM to the intergenic ICR located at the Dlk1-Dio3 locus. Targeted mouse embryonic stem cells allowed to isolate and expand rare cell population that exhibit aberrant methylation, and study the consequences of loss-of-imprinting on normal development. Furthermore, we show that RGM faithfully reflects parent-of-origin methylation inheritance throughout generations, thus facilitating a systematic analysis of methylation dynamics during embryonic development and adults. Taken together, locus-specific readout of endogenous methylation states holds great promise for mechanistic studies with potential broad implications for the field.

DOI: 10.1530/repabs.3.S003

SRF-SRB Exchange Lecture

S004

Progesterone control of regulatory T cell phenotype and abundance

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The fetus is antigenically distinct from the mother and therefore the maternal immune system must establish immunological tolerance towards the fetus to support pregnancy. Fetal-maternal tolerance is primarily mediated by a specialised subset of $CD4^{+}$T cells known as regulatory T (Treg) cells. Absence or reduced function of Treg cells at embryo implantation causes infertility in mice and is implicated as a cause of reproductive disorders in women. The importance of adequate Treg cell responses during pregnancy is well recognised, however the factors which control the strength and quality of this response are not defined. The pregnancy hormone, progesterone (P4), is known to have potent immunosuppressive activity. To investigate the effects of P4 on Treg cells during pregnancy, mated female mice were administered low doses of the P4 antagonist, RU486, in the peri-implantation period. Flow cytometry analyses showed RU486 treatment resulted in decreased proportions of total Treg cells and increased proportions of IFNγ-producing Treg cells in the uterus-draining para-aortic lymph nodes. In vitro, P4 was found to repress IFNγ expression in Treg and T effector cells cultured under Th1-, Th17- and non-polarising conditions. Treg cells from mice with a null mutation in the nuclear progesterone receptor (nPR) also responded to P4 with attenuated IFNγ production, indicating the observed P4 effect was not mediated by nPR. Finally, using a membrane impermeable form of P4, we found Treg cells to be capable of binding P4 at the membrane. These findings suggest a non-classical mechanism for direct P4 action on Treg cells, potentially through membrane P4 receptors (mPRs). Collectively, our work demonstrates that P4 is a regulator of Treg cell abundance and cytokine production, which may be important in the establishment and maintenance of competent maternal tolerance during pregnancy.

DOI: 10.1530/repabs.3.S004

Symposium 2: New roles for old signalling pathways

S005

Leptin revisited: Novel neurohormonal and molecular mechanisms for the reproductive roles of leptin

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The adipose hormone, leptin, which was identified in 1994, has been universally recognized as an essential metabolic signal that transmits information about the magnitude of body energy reserves to the brain centers controlling body weight homeostasis and energy expenditure. In addition, leptin serves a fundamental function as integrator of metabolism and other key bodily systems, ranging from immune responses to the reproductive axis. On the latter, leptin is known to metabolically gate reproductive activation at puberty and fertility, acting mainly as a permissive factor; namely, threshold leptin levels are needed for puberty to proceed and for the maintenance of reproductive competence. However, we have become aware in recent years that the mode of action of leptin for its effects on the reproductive axis is multi-faceted, and includes permissive and inhibitory actions, involving numerous pathways, at different levels of the hypothalamic-pituitary-gonadal system. In this talk, we will present a summary of recent advancements in our knowledge about the mechanisms whereby leptin transmits metabolic information to the reproductive axis, with special attention to its mode of direct or indirect regulation of various brain neuropeptide systems, including kisspeptins, neurokinin B and melanocortins, as well as the central roles of key cellular energy sensors. In doing so, we aim to provide an updated view of the mechanism of action of this essential physiological and pathophysiological regulator of puberty and fertility.

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S006

PI3K regulation of dormant follicle activation

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PI3K is a family of enzyme involved in cellular functions such as cell growth, proliferation, differentiation, motility, survival and intracellular trafficking. The PI3K-Akt signaling pathway negatively is regulated by PTEN. Recently, we developed the method for activation of dormant follicles by using *in vitro* culture of ovarian fragments treated with PTEN inhibitor and PI3K activator (IVA, *in vitro* activation). We applied this approach to infertility treatment of patients with primary ovarian insufficiency (POI). POI patients are infertile due to a lack of follicle growth and ovulation; oocyte donation is the only effective treatment option. We performed laparoscopic surgery to remove ovaries. Ovaries were cut into strips and vitrified. After thawing, the strips were fragmented into small cubes and cultured with PTEN inhibitor and PI3K activator. After two days culture to activate dormant residual follicles, the cubes were autografted under laparoscopic surgery beneath serosa of Fallopian tubes. Follicle growth was monitored via transvaginal ultrasound and serum estrogen levels. With estrogen replacement therapy to maintain normal levels of LH together with estrogen, patients were treated with FSH/HMG for up to 4 weeks followed by hCG when preovulatory follicles were found. Mature oocytes were then retrieved and fertilized before cyropreservation of four-cell stage embryos. Patients received hormonal treatments to prepare the endometrium for implantation followed by transferring of thawed embryos. We enrolled 37 patients into the IVA clinical study Histological analyses in ovaries after laparoscopic ovariectomy revealed that 20 of 37 patients contained residual follicles. Among 20 patients, nine patients achieved successful follicle growth. We could obtain mature oocytes from seven patients, and achieved the successful pregnancy following IVF-ET in three patients, and two healthy babies were delivered from two patients. In this presentation, I will introduce our basic and clinical studies of IVA and some recent progress in the IVA approach.

DOI: 10.1530/repabs.3.S006

S007

AMH and INSL3: 'Testicular' factors with emerging intra-ovarian roles

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Anti-Mullerian hormone (AMH) is a testicular Sertoli cell product belonging to the TGF-beta superfamily responsible for regression of the Mullerian ducts in the male foetus. It plays a pivotal role in sexual differentiation of the internal genital ducts. The existence of AMH was first postulated by Alfred Jost in the 1950s and was long considered to be a foetal testis-specific factor. However, it has since emerged that AMH and its receptors are also expressed by the post-natal ovary and play important roles in the negative regulation of preantral follicle development and FSH-induced steroidogenesis at later follicle stages. In a clinical context, circulating AMH levels are closely linked to antral follicle count estimated by ultrasonography and serum AMH levels are increasingly used as a proxy for 'ovarian reserve' in patients undergoing fertility evaluation/treatment. Another factor first identified in the foetal testis, insulin-like peptide 3 (INSL3), has long been recognised for its role in the trans-abdominal phase of testicular descent. INSL3 secreted by Leydig cells promotes development of the caudal genital ligament (gubernaculum) that fixes the testis to the inguinal region, while androgen inhibits development of the cranial suspensory ligament that would otherwise retain the gonad in a cranial position close to the kidney. Knockout of INSL3 or its receptor (RXFP2) in mice results in cryptorchidism with the testis remaining in an ovary-like position. More recently, INSL3/RXFP2 signaling has been implicated in regulation of ovarian function, with theca cells being a main site of expression. INSL3 signalling appears to modulate ovarian androgen production since knockdown of either INSL3 or its receptor (RXFP2) in bovine theca cells inhibits androgen biosynthesis while exogenous INSL3 can raise androgen secretion. The inhibitory action of bone morphogenetic proteins on thecal androgen production may be mediated by reduced INSL3-RXFP2 signalling. Moreover, circulating INSL3 (and AMH) levels are raised in women with polycystic ovarian syndrome reinforcing a positive association with ovarian androgen production. These aspects of AMH and INSL3 action will be discussed in this presentation.

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SRF New Investigator Award Lecture

S008

Genetic and environmental determinants of male reproductive health

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A wide variety of genetic and environmental factors have been proposed to affect male gonadal development and function during fetal life with impacts on subsequent reproductive health. This includes genetic mutations that may result in disorders of sex development (DSD), and environmental exposures to agents such as plasticizers, synthetic oestrogens and analgesics. Much of the data on the specific effects of these genetic mutations and exposures on gonadal development and function are based on studies conducted in rodent models; however, confirmation of such findings in human-relevant model systems are lacking.
We have developed model systems to determine the effects of genetic manipulation and environmental exposures on human fetal testis development and function. Using a xenograft system designed to reproduce normal fetal testis development and in-utero hormonal environment, our results demonstrate important species differences in the effects of exposure to chemicals such as di-*n*-butyl phthalate (DBP) and diethylstilboestrol (DES) in terms of testosterone production, a key determinant of male reproductive development during fetal life. We have also demonstrated that exposure to analgesics, such as paracetamol, result in a significant reduction in testosterone production and also impact on germ cell development in the human fetal testis. Importantly, these effects are apparent at therapeutic levels of exposure using a standard therapeutic regimen. In addition, we have developed this system to model the effects of known and novel genetic mutations associated with DSD on gonad development during fetal and early postnatal life.
Our work, demonstrates the importance of choosing appropriate model species, experimental systems and exposure regimens to determine the potential impact of genetic mutations and environmental exposures on gonadal development during fetal life which will ultimately determine male reproductive function during adulthood.

DOI: 10.1530/repabs.3.S008

SSR New Investigator Lecture

S009

Epigenetics of the male germline from stem cells to sperm

Satoshi H. Namekawa
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The cellular identity of germ cells, the only heritable lineage to the next generation, is distinct from those of somatic lineages. The somatic program is largely suppressed in male germ cells which retain unique cellular identity, passed on to the compacted sperm, and give rise to a totipotent zygote after fertilization. We recently demonstrated that a few thousand genes commonly expressed in somatic lineages and spermatogenesis-progenitor cells (termed somatic/progenitor genes) undergo repression in a genome-wide manner during late stages of the male germline, and identify underlying mechanisms. SCML2, a germline-specific subunit of a Polycomb repressive complex 1 (PRC1), establishes the unique epigenome of the male germline. In the stem cell phase of spermatogonia, SCML2 works with PRC1 and promotes RNF2-dependent ubiquitination of H2A, thereby marking somatic/progenitor genes on autosomes for repression. This repression of somatic/progenitor genes during meiosis and postmeiosis is associated with formation of a novel class of bivalent domains. We infer that the novel bivalent domains allow for the recovery of the somatic/progenitor program after fertilization. Our results uncovered that bivalent H3K27me3 and H3K4me2/3 domains are not limited to developmental promoters (which maintain bivalent domains that are silent throughout the reproductive cycle), but also underlie reversible silencing of somatic/progenitor genes during the mitosis-to-meiosis transition in late spermatogenesis.
Importantly, during spermatogenesis, mechanisms of epigenetic regulation on sex chromosomes differ from autosomes because of meiotic sex chromosome inactivation that is regulated by DNA damage response pathways. X-linked somatic/progenitor genes are suppressed by meiotic sex chromosome inactivation without deposition of H3K27me3. Furthermore, SCML2 also independently prevents RNF2-dependent ubiquitination of H2A on sex chromosomes during meiosis, thereby enabling unique epigenetic programming of sex chromosomes for male reproduction. Taken together, our genome-wide studies reveal epigenetic principles during the mitosis-to-meiosis transition in spermatogenesis.

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SRF Distinguished Scientist lecture

S010

Making a good egg

John Carroll
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A mature fertile oocyte is the foundation of successful embryo development. This central role in the propagation of the species renders the oocyte a focus for research in fundamental cell biology as well as in clinical research, where understanding oocyte biology provides new insights into the treatment of infertility.
Making a good egg requires many critical processes including dramatic changes in the properties and volume of the cytoplasm, cell-cell interactions, highly regulated cell-cycle progression, establishing and maintaining polarity and the ability to undergo fertilization. All of these processes are designed to provide the necessary maternal cytoplasmic and genomic contributions to the subsequent embryo. Abnormalities in the oocyte cell cycle can lead to the arrest of the oocyte at an immature state or, if the oocyte progresses, the formation of an embryo that contains the wrong number of chromosomes (aneuploidy). The frequency of aneuploidy increases exponentially with maternal age and accounts for much of the reported age-related increase in early embryo loss, miscarriage, infertility and Down's syndrome.
Successful progression through meiosis is dependent on the correct function of key cell cycle regulators that ensure timely progression through the first and second meiotic divisions. Our research has focussed on understanding the mechanisms of how these meiotic divisions are controlled as well as how they are integrated with oocyte polarity and mitochondrial function. Why these processes go wrong with maternal age and more importantly, whether it is possible to circumvent these issues is key to ensuring the making of a healthy viable oocyte.

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Symposium 3: Risks and opportunities in wildlife reproduction

S011

Contraception in domestic animals

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Domestic dogs and cats, whether feral or owned, cause the death of millions of wildlife animals every year either by hunting and killing or through disease transmission. The reproductive rates of both species are very high and the current approach of surgical sterilization is too slow and ineffective to have a significant impact on feral cat and dog numbers. The ideal product would be a single dose injectable that causes permanent sterility for cats and dogs of both sexes and that could be administered to large numbers of animals in a short time.
Immunocontraception GnRh is a key instigator of both male and female reproduction. The development of delivery systems for continued life long stimulation of antibodies through injectable virus particles is being developed. The virus imbeds itself in muscle cells and produces lifetime antigens. Further reproductive hormones such as Kisspektin (2), FSH, LH or AMH may also be targeted.
High dose/long-term GNRH agonist Constant supply of GnRH leads to down-regulation of the receptors and a complete cessation of the reproductive system in both the male and female. The already licenced drug Deslorelin works on this principle for 6–12 months. Attempts are being made to develop new devices to hold and release medication over much longer periods of time.
Cytotoxins delivered to specific sites killing cells that are essential for reproduction could be used. The toxin is conjugated to a specific antibody that binds to the target cells. The problem in reproduction is that the target cells are either in the hypothalamus or the pituitary gland and have to cross the blood-brain barrier.
Gene silencing/gene therapy A specific protein that suppresses reproduction has to be identified. Delivery of the gene via a virus into the cell for the production of the protein must then be achieved. Gene silencing works with small RNA fragments that are incooperated into the cell's DNA to turn off certain genes (3). This has not yet been attempted with reproductive genes, but in other human diseases.

DOI: 10.1530/repabs.3.S011

S012

Molecular mechanisms of reproductive disruption in fish

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Over the last 30 years concerns have risen about the potential for environmental chemicals to cause reproductive effects on wildlife via disruption of endocrine signaling pathways. There are now hundreds of chemicals known to disrupt the endocrine system and a wide range of examples of adverse effects on wildlife, including the feminisation of fish in UK rivers, egg shell thinning in birds, demasculinisation of reptiles and imposex in mollusks.
The aquatic environment acts as a sink for contaminants, and this, together with the increased production of existing and of novel chemicals, results in increased risk of exposure for aquatic organisms, including to reproductive toxicants. There is a significant lack of understanding of the global mechanisms of toxicity of environmental chemicals, alone or in combination with other stressors. This knowledge is essential to build predictive adverse outcome pathways that can support effective management strategies to protect populations at risk.
We have employed transcriptomics to investigate the mechanisms of toxicity of a range of endocrine disrupting chemicals potentially impacting on fish populations, including natural oestrogens and pesticides. We identified conserved and novel pathways of effect for a range of chemicals of interest, including the suppression of cholesterol biosynthesis by linuron, a pesticide commonly found in the aquatic environment. We also demonstrated that Bisphenol A, a component of plastics with weak oestrogenic activity, caused reproductive toxicity in breeding zebrafish, decreased *dnmt1* transcription, and reduced global DNA methylation, as well as inducing changes in the promoter methylation and transcription of genes regulating reproduction. Together, these datasets are helping to build a more comprehensive understanding of the molecular mechanisms of effect of chemicals with suspected endocrine activity. These data are supporting the development of adverse outcome pathways to be used in the management of chemical pollution, in order to protect wildlife and human health.

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S013

Novel technologies in screening zoo and exotic animals

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Initially, zoo-based research was dominated by considerations of husbandry, but more recently new ideas, particularly the use advanced imaging techniques combined with assisted reproduction technologies (ART) have been incorporated. Progressive global habitat destruction and fragmentation is causing dramatic population declines and even the extinction of many threatened species. The complex management of captive populations involves the maximization of genetic variation. Thus requires the exchange of individuals between breeding institutions. In general, animal movements include a (i) high risk of disease transmission, (ii) stress-induced infertility or partner incompatibility as well as (iii) high financial and logistic efforts. The use of ART eliminates the problems of distance and time. However, there are several operative problems before ART can be successfully applied in non-domestic species. Due to unknown reproductive status and often incomplete knowledge of the reproductive anatomy imaging modalities play a crucial role in the process of development new ART technologies as well as in the selection process of the best potential breeding partners.

Main imaging techniques applied are ultrasonography and computed tomography besides thermography and MRI. Our patient sizes range from several tons (elephants, rhinos) to few grams (shrew) requiring scan frequencies of 2.0 to 80 MHz. They can live in water (moray eel, dolphins) or underground (naked mole rats) and need environmental temperatures during the screening procedures ranging from 10° to 30° Celsius (tuatara, desert varanus). Customized equipment originally developed for human beings or livestock can't directly applied to exotic species. The priority for the development of novel ART instruments and technologies should focus mainly on non-invasive or minimal-invasive procedures. In this context it is important that ART procedures correspond with the general guidelines of animal welfare.

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Oral Communications

Oral Communications 1: Ovary

O001

Mice with follicular premature ovarian failure at 3 months of age become a follicular by 1 year of age

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Introduction
Premature ovarian failure (POF) is a reproductive disorder which causes defects within the ovaries, normally leading to infertility. The disorder affects 1% of women under the age of 40 with 75% of cases with no known cause, indicating the need for more research. The Double Mutant (DM) mouse model with oocyte-specific deletion of C1galt1 and Mgat1 exhibit follicular POF at 3 months of age. Therefore, this study aimed to investigate the effect of ovarian dysfunction on the primordial follicle pool of one year-old DM and Contol mice.
Methods
This study was approved by the Local Ethical Review Panel (University of Oxford). Deletion of these genes occurs at the primary stage of follicle development due to deletion of floxed genes by a ZP3Cre transgene. Ovaries from Control and DM mice at 1 year of age were collected, fixed, paraffin embedded and sectioned. Every 10th section was stained with hematoxylin and eosin and the number of primordial follicles quantified. A Mann-Whitney U test was carried out to compare the numbers of primordial follicles in Control and DM ovaries.
Results and Discussion
The quantity of primordial follicles within the DM mouse ovaries was significantly reduced compared to Control mouse ovaries (95.586.06 vs 4.257.18, $P<0.05$, $n=4$, Control, $n=4$ DM). These results reveal that ovarian dysfunction within ovaries with follicular POF modifies primordial follicle development since deletion of the genes occurs after primordial follicle activation, at the primary stage of follicle development. This result reveals the profound effect that normal follicular function has on maintaining the primordial follicle pool and therefore it is possible that all follicular POFs result in afollicular POF due to modified paracrine signalling.
This study was partially funded by Nuffield Department of Obstetrics & Gynaecology.
DOI: 10.1530/repabs.3.O001

O002

The effect of folic acid supplementation during the juvenile pubertal period or adulthood on gene expression in the ovary

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Introduction
Women with mutations in BRCA1 gene have an increased lifetime risk of ovarian cancer. Many studies but not all have shown that an adequate folate intake is protective against many cancers including ovarian, but recent studies have shown that high levels of folic acid (FA) supplementation can promote cancer risk. The aim of this study is to determine FA impact on the expression of cancer related genes in the ovary such as OCT4 a pluripotency factor, BRCA1 related to DNA repair and the chromatin modifying enzyme EZH2.
Methods
Juvenile (4 wks old) or adult (10 wks old) female C57BL/6 mice were fed for 4 weeks with normal (1 mg/Kg), high (5 mg/Kg) or supramaximal (20 mg/kg) doses of FA and then kept on maintenance diet until 4 or 12 weeks after supplementation. Quantitative RT-PCR was performed to determine the mRNA levels.
Results and Discussion
FA supplementation during the juvenile period led to an increase in Oct4, Brca1 and Ezh2 expression immediately after the treatment. However, those effects did not persist after the end of supplementation. In contrast, FA during adulthood led to an increase in Oct4, Brca1 and Ezh2, which persisted 4 weeks after the end of supplementation. These results show that FA affects the expression of cancer related genes in the ovary, but the effects are dependent on the dose and time of supplementation. Future work is needed to identify histological alterations on ovaries and if FA could modify the quality and/or quantity of ovarian follicles.
DOI: 10.1530/repabs.3.O002

O003

PLCz-induced Ca2+ oscillations are enhanced after germinal vesicle breakdown during mouse oocyte maturation

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Introduction
Mature mouse oocytes, arrested at meiotic metaphase II (MII), are activated by a series of Ca^{2+} oscillations caused by sperm-specific phospholipase C zeta (PLCz) which has been shown to localize in cytoplasmic vesicles. The substrate for PLCz, phosphatidylinositol 4,5-bisphosphate (PIP_2), is also present in cytoplasmic vesicles in MII oocytes. Throughout oocyte maturation there is reported to be a gradual increase in sensitivity to PLCz and $InsP_3$ -Ca^{2+} oscillations, which has been attributed to increased Ca^{2+} store content or $InsP_3$receptor sensitivity. Here, we have examined the sensitivity of maturing oocytes to PLCz-induced Ca^{2+} oscillations in relation to the appearance of PIP_2 vesicles.
Methods
Mouse oocytes were collected from culled female mice and maintained in M2 media with, or without, IBMX. Oocytes were microinjected with PLCz-luciferase cRNA and a dextran-linked fluorescent Ca^{2+} dye. Chemiluminescence and fluorescence were measured in oocytes maintained on the heated stage of an epifluorescence microscope equipped with an intensified CCD camera. Immunostaining was carried out on similarly-treated, fixed and permeabilized oocytes.
Results and Discussion
Injection of luciferase-tagged PLCz RNA showed that cytoplasmic PLCz expression of up to ∼35-fold that which is effective in MII oocytes, does not trigger Ca^{2+} oscillations in 41/49 GV oocytes. However, after germinal vesicle breakdown (GVBD), 10/10 oocytes expressing similar PLCz levels to those active in MII oocytes displayed Ca^{2+} oscillations of reduced amplitude. This marked increase in sensitivity to PLCz correlated with the appearance of PIP_2-positive cytoplasmic vesicles that were evident after GVBD. Immunostaining of MII oocytes suggested that PLCz co-localized with markers of Golgi, which is known to fragment during M-phase. These data suggest that the appearance of PIP_2 in cytoplasmic vesicles after GVBD may also sensitize mature MII oocytes to sperm-induced Ca^{2+} oscillations at fertilization.
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O004

Oocyte maturation arrest in endometriosis is caused by elevated levels of Reactive Oxygen Species and enforced via a DNA Damage Response and the Spindle Assembly Checkpoint pathway

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Introduction
It has recently been demonstrated that mouse oocytes respond to DNA damage by arresting in Meiosis I. This arrest has been shown to require the activity of the Spindle Assembly Checkpoint (SAC) and the DNA Damage Response (DDR). Given oocytes lack the equivalent of a G2/M checkpoint for DNA damage, the SAC response may be critical to defend against formation of DNA damaged embryos. It is currently unknown whether the DDR SAC pathway is sensitive to physiological or even pathological levels of DNA damage. We investigate the effect of endometriosis, a disease affecting ∼10% of women of reproductive age, and associated with elevated Reactive Oxygen Species (ROS) and reduced fertility, on oocyte maturation.
Methods
Here we expose mouse oocytes to follicular fluid (FF) from patients with or without endometriosis and assess their ability to complete maturation. We measure ROS and DNA damage in the oocytes and use kinase inhibitors and antisense knockdown to elucidate the pathways involved.
Results & Discussion
We found that FF from patients with endometriosis, but not control FF, elevated ROS and DNA damage in the oocyte, which led to a SAC mediated metaphase I arrest. FF from patients with endometriosis activated ATM kinase, indicating the involvement of the DNA Damage Response. Oocyte maturation could be rescued by blocking ROS, suggesting this is the primary trigger for arrest. We demonstrate for the first time that meaningful levels of ROS and DNA damage can trigger DDR-SAC arrest in oocytes and also provide explanation for the subfertility associated with endometriosis. Furthermore, the pathway proposed provides pharmacological targets and could inform future clinical practice.
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O005

A Positive Feedback Loop between Hypoxia and miRNA-210 Augments Endothelin 2 in Human Granulosa Cells

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Introduction
Hypoxia and Endothelin-2 (EDN2) peak simultaneously during early corpus luteum (CL) formation, suggesting causal relationship. Indeed, hypoxia inducible factor-1 alpha (HIF1A) is a strong stimulator of EDN2 in granulosa–lutein cells (GLCs). Knockout of EDN2 resulted in un-ruptured follicles that failed to develop into CL, suggesting that EDN2 plays essential roles during ovulation. In agreement, EDN2 was low in GLCs of women with PCOS, characterized by ovulatory dysfunction. Hypoxia also induce miR-210, a prototypic hypoxiamiR. However, whether miR-210 affects EDN2 mRNA is unknown yet. We explored here the molecular interactions between miR-210 and EDN2 in several cell models.
Methods
Immortalized or primary GLCs from normal or PCOS women undergoing IVF were studied. Hadassah Review Board approved the study (HMO-0110-09) and women gave informed consent. Cells were incubated in hypoxic and normoxic conditions, miR-210 levels were manipulated and genes were silenced using siRNA. mRNAs and miR-210 were measured by qPCR. HIF1A protein was determine by Western blot.
Results and Discussion
miR-210 and EDN2 were closely related *in vivo* and *in vitro*: hypoxia and miR-210 overexpression both increased EDN2, while miR-210 inhibition reduced EDN2 in immortalized GLC. Also in GLC from PCOS patients, low miR-210 and EDN2 were noted. Furthermore, HIF1A-silenced cells, with decreased EDN2, similarly exhibited lower miR-210. Glycerol-3-phosphate dehydrogenase 1-like (GPD1L) was identified as a gene target of miR-210 lowering its levels either by miR-210 overexpression or siRNA knockdown, increased HIF1A protein and EDN2 levels. Together, these results propose a positive feedback regulatory loop, where miR-210 induced by hypoxia (via HIF1A) lowers GPD1L which further elevate and maintain HIF1A protein and EDN2 levels. This feed forward loop is expected to boost EDN2 in hGLCs exposed to hypoxia during ovulation. On the other hand, reduced miR-210 in PCOS might interrupt the loop, decrease EDN2 and impair ovulation.

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O006

Post-partum endometritis: Its negative impact on luteal vascularisation, progesterone content and steroidogenic enzyme expression in the cow

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Introduction
In dairy cows, post-partum endometritis caused by Gram negative bacteria (e.g. E. coli) adversely affects follicular function and is associated with subfertility. However, there is limited information of the effects of endometritis on the corpus luteum (CL). Recently, we showed that LPS dose-dependently decreased luteal endothelial cell (EC) network formation *in vitro*. The hypothesis tested was that CL from cows with endometritis would have reduced vascularisation and steroidogenic capacity.
Methods
Mid-luteal phase bovine ovaries were collected from cows with either no signs of endometritis (control, $n=3$) or presence of purulent endometrial discharge ($n=3$) confirmed macroscopically and histologically. Luteal sections were either snap frozen or fixed in Bouin's. Ether-extracted progesterone content was determined by ELISA. Immunohistochemical analysis determined the degree of vascularisation (von Willebrand Factor) and pericyte coverage (smooth muscle actin SMA). Western blots assessed STAR, HSD3B, P450SCC and SMA protein expression (normalised to Histone H3). Groups were compared by *t*-tests.
Results and discussion
CL from cows with endometritis were slightly smaller ($P<0.05$) with reduced luteal progesterone content (1.2-fold, $P<0.05$). Control CL contained extensive vascularisation with steroidogenic cells largely adjacent to endothelial cells. This vascularisation (3-fold, $P<0.01$) and pericyte coverage (4-fold, $P<0.001$) was much lower in endometritic cows. Western blot confirmed that SMA protein expression was lower (6-fold, $P<0.001$). Quantification of Western blots clearly showed lower STAR (2-fold), HSD3B (8-fold) and P450SCC (3-fold) protein levels in cows with endometritis (all $P<0.01$). In summary, cows with endometritis had smaller CL with reduced progesterone content. This was associated with decreased expression of progesterone synthesis proteins. Dramatically attenuated luteal endothelial cell area and pericyte coverage suggest that the luteal vasculature might be particularly sensitive to uterine-derived bacterial endotoxins.

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Oral Communications 2: Early Development 1

O007

Cyclical DNA methyltransferase and histone deacetylase expression across multiple timescales in the uterus of the Siberian hamster (Phodopus sungorus)

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Introduction
It is becoming clear that epigenetic modifications, such as DNA methylation, exhibit dynamic and reversible changes. Our understanding of a role for epigenetic modifications for timing biological rhythms is in its infancy. It has recently been found that DNA methylation in the hypothalamus plays a role in regulating the internal representation of seasonal timing. Here we tested the hypothesis that epigenetic modifications are also responsible for controlling reproductive rhythms across a number of timescales in peripheral reproductive tissues.
Methods
Using a seasonally breeding animal model, the Siberian hamster (Phodopus sungorus), we examined the naturally occurring seasonal and estrus variation in mRNA expression of DNA methyltransferase (dnmt) and histone deacetylase (hdac) expression in the uterus.
Results and Discussion
SD conditions induced reproductive involution and a significant increase in uterine dnmt3a and hdac2 expression. One-way ANOVA revealed a significant difference in dnmt3a expression across the estrus cycle levels decrease during oestrus. Ovariectomised hamsters treated with a single bolus of estrogen and progesterone were found to have significantly lower uterine dnmt3a expression. Conversely, there was a significant increase in hdac1 and hdac3 during oestrus. These data provide novel and robust evidence that dnmt3a expression is dynamic across a number of different timescales. We propose that variation in DNMT3a is involved in the local timing of reproductive physiology in key tissues. These data have significant implications for our understanding of the potential effects of DNA methylation for fertility in a rodent species with direct applications for human reproductive health. Uncovering the mechanisms that underlie this natural pattern could have a significant impact for developing effective long-term male contraceptives. We suggest that epigenetic modifications are involved in molecular timing across multiple timescales and may represent an evolutionarily ancient clock mechanism. (This work was funded by an SRF Vacation Scholarship and the University of Aberdeen.

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O008

Mouse blastocyst implantation in an *in vitro* model is promoted by early apposition with the uterine epithelium and by hyperosmolar stress

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Implantation failure remains a bottleneck in assisted reproduction treatments (ARTs), as only ∼25% of treatment cycles result in a live birth. During the early stages of implantation, endocrine and embryonic paracrine signals prime the receptivity of the uterine luminal epithelium (LE) before the trophectoderm (TE) of the blastocyst-stage embryo can mediate attachment and subsequently invade the uterine epithelium and stroma. We have developed an *in vitro* model using the human endometrial adenocarcinoma Ishikawa cell line with mouse blastocysts. Weak and reversible adhesive interactions were observed during co-culture of embryonic day (E) 4.5 blastocysts with Ishikawa cells over 28 h before embryos

attained stable attachment over the following 20 h, progressing to breach the Ishikawa cells by 48 h. This process was steroid hormone-independent. Embryos that were initially cultured in the absence of Ishikawa cells (from E4.5-5.5) attached with the same kinetics as co-cultured embryos, but their ability to breach the epithelial layer was strongly impaired this effect was dependent on contact between blastocysts and Ishikawa cells as revealed by transwell co-culture experiments. Hyperosmolarity is a clinically relevant and experimentally well-defined embryonic stressor. Remarkably, repeated hyperosmolar stress (400mOsm osmolarity increase) at E5.5 in the absence of prior co-culture promoted embryonic breaching of the Ishikawa cells to levels seen with co-culture from E4.5. Furthermore, pharmacological inhibition revealed this effect to be dependent on signalling through the stress-activated protein kinase JNK. Stress is known to induce differentiation of embryonic cells, leading us to hypothesise that TE differentiation is required for breaching of the LE, and that both apposition with the LE and stress can promote implantation. These findings have clinical implications for ART as well as biological importance in understanding early pregnancy. Moreover, demonstrating that stress impacts this epigenetically critical developmental period has epidemiological relevance in line with the developmental origins of health and disease hypothesis.

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O009

A novel role of Adam 10 in tight junction formation during mouse preimplantation development

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A Disintegrin and Metalloprotease Domain-containing protein 10 (ADAM10) is a cell surface protein with a unique structure possessing potential adhesion and protease domains. However, the role of ADAM10 is not known during early embryo development. In this study, we investigated the expression patterns and biological function of Adam10 in mouse preimplantation embryos. The transcription level of *Adam10* increased from the two-cell stage onward. Immunostaining revealed that Adam10 was localized to the apical region of the outer cells in blastocyst embryos. Knockdown (KD) of *Adam10* using siRNA significantly affected blastocyst development. FITC-dextran uptake assay in *Adam*10 KD showed defect of paracellular permeability sealing, and ICC demonstrated aberrant localization and expression of TJ complex constituents. Particularly, Cxadr is mainly detected in the nuclei of blastomears at the blastocyst stage in the KD embryos rather than apical region. However, TJ associated genes were not changed at the transcription level. An *in situ* proximity ligation assay demonstrated direct interaction of ADAM10 with CXADR, supporting the involvement of ADAM10 in TJ assembly. In conclusion, our findings strongly suggest that ADADM10 is important for blastocyst formation rather than compaction in mouse preimplantation development. This work was supported by Next-Generation BioGreen21 Program (PJ011213), Rural Development Administration (RDA), Republic of Korea.

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O010

Investigating of the role of Tribbles-2 protein in mammalian embryo implantation

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Introduction
Embryo implantation is a complex and highly regulated process. Toll-Like receptors (TLRs) play a strategic role in recognition of pathogens in female reproductive tract. Activation of TLRs in endometrial cells at the time of embryo implantation appears to have negative effect on implantation. Tribbles-2 (Trib2) protein is members of tribbles family of psuedokinase proteins, modulating TLR5 signal transduction pathway.Tribbles-2 knockout female mice are infertile. Accordingly, we hypothesized that Trib2 regulate embryo implantation via controllingTLR5 signalling pathway.
Methods
To investigate Trib2 protein involvement in embryo implantation, wild-type mouse embryos were transferred into the oviducts of Trib2 null, Trib2 heterozygotes and wild-types. Furthermore, the desired combination of the functional TLR5 signalling pathway and the functional Trib2 protein in different human endometrial cell-lines (RL95-2, Ishikawa and Ishikawa 3H12) and an epithelial cell-line (HEK293T) was compared. Finally, to test Trib2 importance for embryo implantation in human, we used an *in vitro* binding assay based on a 2D co-culture of endometrial and trophoblast (JAr) cells.
Results and Discussion
No embryo successfully implanted in the uterine horns of Trib2 null females indicating the involvement of Trib2 protein in the implantation process. None of the endometrial cells tested, showed the combination of a functional TLR5 signalling pathway and a functional Trib2 protein. In contrast, HEK293T cells had both these features. Knock down of trib2 gene expression using siTrib2 led to a significant further reduction in adhesion of Jar spheroids to the HEK293T monolayer in the presence of TLR5 agonist, Flagellin (P value < 0.0001). Our results demonstrated that Trib2 is essential for successful embryo implantation in mice. Though, HEK293T cells are from non-reproductive origin, the endogenous expression of both TLR5 and Trib2 proteins in this cell-line, made it the optimum model for inspecting the Trib2 functions in humans.

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O011

Investigating O-GlcNAcylation in an *in vitro* model used to mimic diabetes, and its effects on implantation

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The incidence of diabetes has increased in recent decades and by 2030, 366 million people worldwide will have the disease a significant proportion of these will be women of reproductive age. Diabetes is known to reduce fecundity and increase the likelihood of early pregnancy loss we propose that the high glucose levels seen in diabetes influence maternal fertility by impairing endometrial receptivity/function as a result of increased flux in the hexosamine biosynthetic pathway (HBP). The HBP integrates cellular nutrient, including glucose, metabolism to produce UDP-N-acetylglucosamine, which is used to O-GlcNAcylate proteins and regulate their function. Here we show that it is possible to manipulate the HBP in the Ishikawa endometrial epithelial cell line by increasing flux through the pathway using high glucose (25 mM) or the HBP intermediate, glucosamine (5 mM), leading to an increase in protein O-GlcNAcylation. In addition, we enhanced Ishikawa cell protein O-GlcNAcylation by inhibiting the enzyme, O-GlcNAcase (OGA), responsible for de-GlcNAcylation pharmacologically, or by siRNA knockdown (82% decrease in protein expression). However, glucosamine, but not high glucose or OGA inhibition, elicited an ER stress response, detected as an initial increase in expression of the transcription factor, XBP1S (5.4-fold), followed by increased GRP78/BiP expression (2.3-fold). The functional consequences of manipulating the HBP were investigated by assessing the attachment of blastocyst-sized (40–100 μm) spheroids of human trophoblast (BeWo cells) to Ishikawa cells ($n=8$). Under control conditions, 75% of spheroids were attached after 30 minutes however, the ability of spheroids to attach to Ishikawa cells was not affected by any of the strategies used to increase endometrial cell protein O-GlcNAcylation. Ongoing experiments are testing the effect of altering HBP flux on embryo implantation competence. These studies will help understand the mechanisms underlying impaired fertility in women with diabetes and will contribute to developing interventions to improve endometrial receptivity and pregnancy success.

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O012

Efficacy of progestogen supplementation in women undergoing assisted reproductive technology treatment: A meta-analysis

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Introduction
Luteal phase deficiency following assisted reproductive technology (ART) programmes has led to progestogen supplementation. However, there is debate over which route of progestogen administration (intramuscular or vaginal) is most beneficial and whether additional treatment with oestrogen further improves pregnancy rates. Thus, a large-scale meta-analysis was performed to investigate

the benefit of these luteal phase support treatments on pregnancy outcome in women undergoing ART.
Methods
Literature searches (Google Scholar, PubMed, Medline and Web of Science) from 1980 to 2015 identified 112 studies ($n=18792$ cycles) with progestogen supplementation via intramuscular or vaginal routes. These were sub-divided by time that supplementation started: at oocyte retrieval [OoR], between OoR and embryo transfer [OoR-ET] or at ET. For comparison of additional oestrogen treatment, 21 studies ($n=1702$ cycles) were identified, which were sub-divided based on start time [at OoR or OoR-ET] and route (intramuscular or vaginal) of progestogen supplementation. The effect of the different treatments on the odds ratio (OR) of a clinical pregnancy was determined by logistic regression analysis.
Results and discussion
Overall, pregnancy rates were greater when progestogen was supplemented via intramuscular ($P<0.01$) versus vaginal routes. Clinical pregnancy rates were greater in the intramuscular progestogen supplementation group at OoR (OR = 1.50, $P<0.001$) and OoR-ET (OR = 1.30, $P<0.001$) but lower at ET, (OR = 0.75, $P<0.05$) compared with vaginal route. Oestrogen treatment increased pregnancy rates when progestogen was supplemented by intramuscular (OR = 1.48, $P<0.001$) and vaginal (OR = 1.33, $P<0.001$) routes. Oestrogen treatment greatly increased pregnancy rates when progestogen supplementation was started at OoR-ET (OR = 1.95, $P<0.001$) but was only marginal at OoR (OR = 1.15, $P<0.05$). Pregnancy rates were greatest when progestogen was administered intramuscularly but only when progestogen supplementation was started at the optimum time. Additional oestrogen treatment further increased pregnancy rates when progestogen was administered intramuscularly or vaginally.

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SRF Post-Doctoral Prize Session

O013

Identification of novel DAZL targets in the human fetal ovary

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Introduction
DAZL (deleted in azoospermia-like) is an RNA binding protein essential for germ cell entry into meiosis and later stages of germ cell maturation, and its absence is associated with infertility in vertebrates. Efforts to study DAZL and its *in vivo* RNA targets have been largely limited to mouse, thus human-specific investigations are required.
Methods
Human DAZL protein was immunoprecipitated from 17 week ovarian lysate bound RNAs were sequenced using the Illumina HiSeq platform. 3′UTR-luciferase assays and polysome profiling were utilised to confirm translational regulation by DAZL of novel target RNAs. RNAi knockdown of Dazl in e13.5 fetal mouse ovary cultures was used to investigate functional consequences of DAZL regulation of newly identified targets.
Results and discussion
Differential gene expression analysis using DESeq2 found 429 RNAs that were significantly enriched by DAZL immunoprecipitation (Padj < 0.01). Bioinformatic analysis revealed the presence of at least one consensus mouse Dazl binding element in the 3′UTR of > 97% of the potential targets, indicating similarities between mouse and human DAZL binding sites. Gene-set enrichment analysis identified the gene ontology meiosis (GO:0007126), with RNAs involved in cohesin establishment and DNA repair, which are novel findings, as well as in synaptonemal complex formation. Increased luciferase activity ($P<0.004$) and polysome profiling ($P<0.05$) demonstrated that DAZL regulates the translation of novel human target RNAs SMC1B, RAD51, TEX11 and SYCP1 via their 3′UTR. Creation of a Dazl hypomorph using RNAi knockdown in e13.5 fetal mouse ovary cultures resulted in decreased expression of target RNAs. Furthermore, Dazl hypomorph oocytes had a smaller nuclear diameter than scrambled RNAi controls after 13 days of culture ($P=0.0047$) ongoing studies are investigating potential impact on meiotic progression.
These data demonstrate novel RNA targets of DAZL in human fetal oocytes and identified pathways by which DAZL may contribute to lifelong oocyte quality in women.
[Supported by grants from the Medical Research Council].

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O014

Etoposide results in follicle loss in the fetal mouse ovary, but does not block the ability of oocytes to progress through prophase I of meiosis

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Introduction
The chemotherapy agent etoposide is a topoisomerase II (topo II) inhibitor, and is considered safe to administer during pregnancy. However, assessment of its effects on the developing ovary, when germ cells are undergoing initiation of meiosis and forming follicles, has been limited. We have investigated this using ovarian tissue culture.
Methods
E13.5 mouse ovaries were cultured for 12 days on an agar block, with etoposide added for the first 6 days of culture at concentrations of up to 150 ng/ml, thus exposing the germ cells for the period prior to follicle formation. Follicle numbers and health were analysed histologically. Immunohistochemistry was used to determine topo IIa localisation in mouse and human fetal ovary, and to examine the progression of cultured mouse oocytes through prophase I of meiosis, by visualisation of Sycp3.
Results and discussion
Etoposide did not block the ability of oocytes to progress through meiosis to the diplotene stage of prophase I (after which oocytes enter meiotic arrest), with around 80% of oocytes having reached that phase of meiosis after six days of culture in both etoposide-treated and control groups. There was however, evidence of a more rapid progression through early meiosis: more germ cells from the etoposide-treated ovaries had progressed from leptotene/zygotene to pachytene stage after 2 days in culture compared with controls (77% vs 47%, $P<0.001$). A dose-dependent reduction of follicle numbers was observed following treatment with etoposide, with a near-complete loss of healthy follicles at the top dose (89.7% loss, $P<0.001$). Topo IIa expression was confined to the germ cells prior to follicle formation in both human and mouse fetal ovaries. Our results show that germ cells can progress through prophase I to diplotene during exposure to etoposide, but their ability to form follicles is markedly impaired.

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O015

The crosstalk between Nodal and Tumor Necrosis factor alpha during luteolysis establishment in the mare

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Introduction
In the absence of pregnancy, the resumption of a new estrous cycle is assured by functional and structural regression of the corpus luteum (CL). We have recently characterized the involvement of Nodal, a morphogen from transforming growth factor beta superfamily, on functional luteolysis in the mare (Galvao et al, 2015 Endocrinology, 157:858–871). Also, tumor necrosis factor α (TNF) has been reported to mediate both functional and structural luteal regression. Herein, we demonstrate the dialogue between these two pathways towards luteolysis promotion in the mare.
Materials & methods
An *in vitro* study with enzymatically isolated luteal cells ($n=6$/group) from mid luteal stage (mid-CL), cultured in T25 culture flasks (5.0×10^6 cells/mL), was performed. Cells were treated for 24 h with: (i) no factor (ii) Nodal (0.1–10 ng/mL) (iii) TNF (10 ng/mL) (iii) prostaglandin (PG) F2α (10^{-7}M); or (iv) luteinizing hormone (LH) (10 ng/ml). TNF was quantified in culture media (ELISA). The mRNA transcription was evaluated by relative quantification real-time PCR (normalization with β-microglobulin- B2MG) and protein expression quantified by western blotting (normalization with β-actin).
Results & discussion
Nodal increased the TNF level in culture media ($P<0.05$), as well as mRNA and protein of TNF ($P<0.05$) and its receptor type I ($P<0.05$). Conversely, the TNF activation of Nodal signalling was supported by a consistent rise in mRNA and protein of Nodal ($P<0.05$) and its receptors Alk4 ($P<0.01$) and Alk7 ($P<0.05$). Ultimately, phosphorylation of Smad3 protein, an intracellular mediator of canonical Nodal signalling activation, by TNF testifies the cross talk between both pathways. The present results suggest the dialogue between TNF and Nodal signalling pathways for luteolytic signal amplification during CL regression in mares.

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SRF Student Prize Session

O016

Exaggerated metabolic changes during puberty precede adult obesity and hyperlipidemia in an ovine model of Polycystic Ovary Syndrome (PCOS)

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Women with Polycystic Ovary Syndrome are at increased risk of developing insulin resistance, obesity and dyslipidemia. Amplified metabolic perturbations during puberty may be a central factor contributing to metabolic phenotype of adult PCOS. Using a clinically realistic ovine model of PCOS we reported hyperinsulinaemia and early fatty liver changes, with no difference in body weight and adiposity, in adolescence.

Here we aimed to further examine metabolic events during transition from adolescence to adulthood. Pregnant Scottish Greyface ewes were treated biweekly with either 100 mg of testosterone propionate (TP) or vehicle control (C) from day 62–102 of gestation. Two cohorts of animals, adolescent 11 months old (C=5 TP=9) and adult 30 months old (C=11 TP=4), were investigated.

During puberty, but not fetal or early life, there was decreased adipogenesis in subcutaneous adipose tissue (SAT) accompanied by decreased circulating concentrations of fibroblast growth factor 21 (FGF21), leptin and adiponectin, and increased concentrations of fasting free fatty acids (FFA) ($P<0.05$) in prenatally androgenized sheep. This was countered by upregulated expression of FFA transporters in liver SLC27A2, SLC27A5, CAV2, FABP4 ($P<0.05$). As young adults, TP-exposed animals had increased body weight ($P<0.05$), increased insulin concentration ($P<0.05$) and FFA levels ($P<0.05$) but with no difference in FGF21, leptin and aidponectin levels. Histological analysis revealed that TP-exposed animals have decreased total number of adipocytes ($P<0.05$) and increased mean adipocyte size in SAT ($P<0.05$).

Altered adipogenesis in SAT of PCOS-like sheep and decreased levels of beneficial adipokines correlate with onset of puberty and hyperinsulinaemia and results in hypertrophy of adult SAT. This consequently lowers capacity of SAT to safely store fat and potentially explain metabolic perturbations observed in PCOS-like female sheep. These provide better understanding into the pathophysiology of PCOS from puberty to adulthood and give opportunities for early clinical intervention to ameliorate the metabolic phenotype of PCOS.

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O017

Derivation and use of mouse embryonic stem cell lines as model for mechanistic analysis of periconceptional developmental programming

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Introduction

The developmental origins of health and disease (DOHaD) concept proposes that maternal environment during pregnancy may influence offspring health and predispose to chronic disease risk in later life. Our previous mouse *in vivo* studies showed that maternal low protein diet (LPD) and advanced maternal age (AMA) programme the preimplantation embryo to adult disease, notably cardiometabolic dysfunction. To test this hypothesis and understand the mechanistic basis of periconceptional programming, we derive mESCs from LPD and AMA models.

Methods

MESCs were derived from blastocysts collected from mothers fed LPD (9% casein) and normal protein diet (NPD, 18% casein) only during E3.5 and AMA model of old (7–8 months) and young (7–8 weeks) dams.

Results and discussion

LPD lines showed reduced derivation efficiency (20%, $P<0.0155$ blastocyst/mother), Nanog: Oct4 expressing cells in outgrowths ($P<0.05$) and increased apoptotic cells ($P<0.05$) than NPDs. Cell signaling activity was similar for Akt, Stat3 and p38 pathways but ERK 1/2 was reduced in activity in LPD lines ($P<0.05$) suggesting reduced MAPK survival signaling may contribute to LPD ESC enhanced apoptosis. Global metabolomics profile of LPD lines depicted altered glucose metabolism, fatty acid homeostasis and ascorbate utilization. These variations indicate stress management strategies of mESCs to combat detrimental effects of LPD induced during (and preserved since) early embryo programming (E3.5). AMA derived lines showed similar embryo and ESC derivation efficiency, male: female ratio, normal karyotype percentage across treatments but reduced cellular proliferation. To evaluate their physiological status, we are analyzing cellular apoptosis, mitotic cycle, gene expression profiles and metabolic pathways critical in development of a healthy metabolism. These mESCs act as models that mimic the inaccessible embryo and fetus within the mother, providing a mechanistic approach to understand the phenotypic changes induced by environmental effects thereby reducing the total number of animals used. (Funded by BBSRC, EU-FP7, EpiHealth, EpiHealthNet).

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O018

FACS-sorted putative oogonial stem cells from the mouse ovary are neither DDX4-positive nor germ cells

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Whether the adult mammalian ovary contains oogonial stem cells (OSCs) is controversial. They have been isolated by fluorescence-activated cell sorting (FACS) using the germline marker DEAD box polypeptide 4 (DDX4), previously assumed to be cytoplasmic, not surface-bound. Furthermore their stem cell and germ cell characteristics remain disputed. We applied a validated protocol to isolate mouse putative OSCs from whole ovarian cell suspensions, then assessed their *in vitro* germline and meiotic progression.

FACS-positive cells were indeed isolated using a DDX4 antibody. By immunofluorescence, they stained positive on their cell surface, but did not express measurable Ddx4 mRNA by PCR. Similarly, adult mouse oviduct showed high levels of immunofluorescence staining for DDX4, but no associated gene expression. A second independent antibody to a larger part of DDX4 was used, and both cell types stained negative. We conclude that the DDX4 antibody used for FACS sorting is not binding to a membrane-bound DDX4, but instead isolates cells through unrelated protein affinity.

The FACS-sorted cells were further interrogated for gene and protein detection of germline and oocyte markers (Prdm1, Dppa3, Ifitm3, Ddx4, Dazl, Pou5f1, Stra8, Nobox, Zp3). Despite them initially not possessing germline identity, they acquired some pre-meiotic markers in culture (Ddx4, Pou5f1), but critically never expressed markers for meiosis or oogenesis. Morphologically they never produced large rounded oocyte-like cells. Furthermore, the cells were not immortal but died within three months post-sorting.

Our results suggest that freshly isolated OSCs are not germ stem cells, and are not being isolated by their DDX4 expression. However it may be that culture induces some pre-meiotic markers. In summary the present study offers weight to the dogma that the adult ovary is populated by a fixed number of oocytes, and that adult de novo production is a rare or insignificant event.

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O019

Immune cell dynamics in endometrial repair and remodelling

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Introduction

The endometrium is one of the few adult tissues that heals repeatedly without scarring. On a monthly basis the endometrium undergoes cyclical episodes of proliferation, degeneration, tissue repair and remodelling in response to the 'injury' inflicted on it during menstruation. In women, endometrial shedding (menses) is the culmination of a cascade of inflammatory signals between uterine stromal cells and immune cells both of which have key roles in endometrial breakdown and repair. In mice, we can induce endometrial shedding and replicate rapid, scar-free healing of the endometrial lining (Cousins et al2014 PLOS ONE). Inflammatory cells play an essential role in tissue breakdown during menses but their role in repair and restoration of tissue homeostasis remains poorly understood. In the current study we investigated immune cell dynamics during endometrial repair and remodelling.

Methods
Menstruation was simulated in vivo in MacGreen® mice, in which cells of the mononuclear phagocyte system express GFP, in order to investigate dynamic changes in immune cell populations during post menstrual endometrial repair and remodelling (8, 24, 48 h after removal of progesterone). Uterine horns were collected for flow cytometry or fixed frozen for immunohistochemistry. Tissue distribution of immune cells was determined by immunohistochemistry GFP+ cell populations were analysed by Flow Cytometry.
Results and Discussion
Immunohistochemistry demonstrated striking spatio-temporal changes in numbers and location of GFP+ cells during endometrial breakdown and repair which peaked 24 h after removal of progesterone. Flow Cytometry revealed a significant influx of GFP+ cells during repair the majority of which were characterised as Gr-1^{+}F4/80^{+}. These data provide the first compelling evidence to support a dynamic role for inflammatory monocytes in endometrial repair and provide the platform for future studies on the role of these cells in scarless healing. (Supported by MRC programme grant to PTKS and an MRC Doctoral Training Grant to PMK).

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O020

Maternal high fat diet (HFD) in the adult offspring brain modifies cell density and neuronal density

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Introduction
An upward trend in maternal obesity is rising every year. Different data suggest that maternal obesity during gestation may have effects on a high risk of children of developing physiological and psychological dysfunctions in later life. Animal models suggest that a maternal high fat diet (HFD) during pregnancy could have enduring consequences on brain structure and development in the offspring. Our aim is to evaluate the effects of maternal HFD on both offspring brain development and neural stems cells (NSCs).
Methods
Female mice were fed different diets from conception: chow diet (CD), HFD throughout gestation and lactation (HFD) or embryonic HFD (Emb-HFD: HFD for 3.5 days, CD thereafter). After weaning, the offspring were maintained on CD. 5 male brains and 6 female brains were collected per group and analysed by immunostaining.
Results and Discussion
We showed an increase in cortical layer thickness (layer 2/3 $P=0.0461$ layer 6 $P=0.0023$) increase in total cortical cell density (layer 2/3 $P=0.0087$ layer 5 $P=0.0266$ layer 6 $P=0.0080$) and reduction in neuronal proportion (layer 2/3 $P=0.0601$ layer 4 $P=0.0025$ layer 6 $P=0.0039$) in the HFD males compared with CD males. Similar results have been found in female offspring brains, with increase cell density (layer 4 $P=0.0058$, layer 5 $P=0.0010$) in the HFD females compared with CD females. Additionally, when NSCs were examined in the subventricular zone, Emb-HFD males showed increased neural stem cells compared to CD males ($P<0.05$). However, there was no significant difference in the density of astrocytes or microglia between male groups. Further work will determine the cells responsible for the increase cell density. Taken together, our data suggests that neurogenesis and brain morphology are altered following maternal HFD and this might result in long term changes in brain architecture. Further research will be important for a better understanding of the effect of maternal HFD on brain development.

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O021

Abstract unavailable.

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Oral Communications 3: Environmental and programming effects on reproduction

O022

Role of miRNAs in the hypoxic regulation of human embryonic stem cells

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Human embryonic stem cells (hESCs) proliferate by self-renewal and hold much promise for regenerative medicine since they have the potential to develop into all cells of the body. In culture, hESCs are difficult to maintain as they have a propensity to spontaneously differentiate. A low, 5% oxygen concentration (hypoxia) promotes hESC maintenance but the mechanisms which regulate this effect are unknown. We hypothesise that changes in environmental oxygen alter the expression of microRNAs (miRNAs) to regulate hESC pluripotency. TaqMan human miRNA arrays (Card A, Applied Biosystems) were performed on hESCs cultured at either 5%, or 20% oxygen and 40 miRNAs were found to be differentially expressed. Bioinformatic analyses of a subset of miRNAs that were down-regulated under hypoxic compared to atmospheric oxygen tensions were predicted to target NANOG, a key transcription factor regulating hESC pluripotency. RT-qPCR was used to confirm a significant down-regulation of these miRNAs. To determine whether these miRNAs regulate NANOG expression, specific pre-miRNAs or negative control pre-miRNAs were transfected into hESCs cultured at 5% oxygen. Using Western Blotting, NANOG protein expression was found to be significantly reduced. Dual-luciferase reporter assays are currently being performed to determine whether these miRNAs bind directly to the NANOG 3′UTR. These data suggest that miRNA expression is altered by environmental oxygen and regulate hESC self-renewal. Funded by the Gerald Kerkut Charitable Trust, the University of Southampton and the Society for Reproduction and Fertility.

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O023

Paternal low protein diet affects fetal growth, placental development and skeletal formation in mice

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Human and animal models have demonstrated the importance of maternal gestational diet for fetal growth, placental function and long-term offspring health. However, the impact of paternal diet on offspring development remains under-investigated. We have demonstrated that a paternal low protein diet (LPD 9% protein), programmes elevated offspring weight at birth, adult adiposity, glucose intolerance and cardiovascular dysfunction when compared to offspring from control normal protein diet (NPD 18% protein) fed male mice. As perturbed weight at birth is a critical indicator of cardio-metabolic disease risk in adult life, our current study investigated the impact of paternal LPD on post-fertilisation development and fetal growth. Male C57BL6/J mice were fed either NPD or isocaloric LPD for 7 weeks prior to mating. Analysis of NPD and LPD E3.5 blastocyst AMPK pathway by PCR array (Qiagen), revealed reduced LPD expression of receptor signalling, signalling cascades, protein synthesis, transcriptional regulation and metabolism genes (17 in total $P<0.05$). In late gestation (E17), fetal and placental weight were increased and decreased respectively in LPD offspring ($P<0.05$). In LPD placentas, reduced labyrinth and increased junctional zone proportions were observed ($P<0.05$), with increased transcript expression of calcium, (Atp2b1), amino acid (Slc38a2) and glucose (Slc2a1, Slc2a4) transporters when compared to NPD placentas ($P<0.05$). In LPD fetal liver tissue, reduced transcript expression of Adipor1, Akt2, Prkaca, Prargc1a and Trp53, ($P<0.05$) were observed, all reduced in LPD blastocysts. Micro-CT analysis of E17 fetal skeletal development revealed significantly increased low-density and decreased high-density bone in LPD offspring when compared with NPD offspring ($P<0.05$). These novel data suggest that paternal diet, at the time of conception, affects blastocyst metabolism, fetal growth, skeletal formation and placental development. Altered blastocyst metabolism and enhanced fetal growth may originate from adaptive mechanisms initiated to ensure short-term survival in the offspring, but which ultimately become maladaptive in adult life.

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O024

Maternal protein restriction around conception alters the foetal mouse brain by reducing the neural stem cells and increasing neuronal differentiation during gestation, which might be associated with the adult behavioural deficits

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Introduction
Maternal malnutrition during pregnancy is detrimental to fetal development and increases the risk of many chronic diseases in later life i.e. neurological consequences such as increased risk of schizophrenia. Previous studies have shown maternal protein malnutrition during pregnancy and lactation compromises brain development in late gestation and after birth, affecting structural, biochemical and pathway dynamics with lasting consequences for motor and cognitive function. However, the importance of nutrition during embryogenesis for early brain development is unknown. We have previously shown maternal low protein diet confined to the preimplantation period (Emb-LPD) in mice is sufficient to induce cardiometabolic and locomotory behavioural abnormalities in adult offspring.
Methods
Using a diet model, female mice were fed different diets from conception to the end of pregnancy: normal protein diet (NPD), low protein diet (LPD) or embryonic LPD (Emb-LPD: LPD for 3.5 days, NPD thereafter). Fetal brains were analysed at three time points in gestation (E12.5, E14.5 & E17.5), with *in vivo* analysis using FACS and immunofluorescence for neural stem cell and neuron markers, and *in vitro* techniques using the neurosphere culture assay. We have also carried out a number of follow up behavioural tests for memory including novel object recognition in adult offspring.
Results & Discussion
We have shown that Emb-LPD and sustained LPD reduce neural stem cell (NSC) and progenitor cell numbers through suppressed proliferation rates in both ganglionic eminences and cortex of the fetal brain at E12.5, E14.5 & E17.5. Moreover, Emb-LPD causes remaining NSCs to upregulate the neuronal differentiation rate in compensation beyond control levels. We have also seen a deficit in short term memory in the Emb-LPD adult offspring. This data is the first to clearly demonstrate that poor maternal nutrition around conception has adverse effects on early brain development, which might be associated with the adult behavioural deficits.

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O025

Maternal dietary protein restriction had no adverse effect on fetal ovarian morphology and germ cell markers on day 65 of gestation in sheep

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Introduction
The continued decline in fertility is a multifactorial problem and has severe adverse impact on a farm's profitability. Under-nutrition during pregnancy can subsequently affect the cardiovascular system, renal function and fertility in the offspring. This study tested the hypothesis that maternal protein restriction would adversely affect fetal ovarian development and number of germ cells.
Methods
Fourteen Scottish Blackface ewes were fed either control (CP $n=6$) or low protein diet (LP $n=8$ 17 vs 8.7 g crude protein/MJ metabolisable energy) from conception to day 65 of gestation when the ewes were euthanised. Fetal ovaries were weighed, fixed in Bouin's and paraffin-embedded. Sections (5 μm) were subjected to histological and immunohistochemical analysis for the oogonia/oocyte markers: OCT4, DAZL and VASA. The oogonia/oocyte counts were determined by image analysis and groups compared by *t*-test.
Results and discussion
Fetal ovaries tended to be smaller in the LP (201.2 mg) versus CP (252.6 mg) diet ($P=0.08$). There was no difference in ovarian histological morphology between the two groups. There was an abundance of OCT4, DAZL and VASA-positive cells in the fetal ovarian cortex on day 65 of gestation. The numbers of OCT4, DAZL, and VASA-positive cells within the ovigerous cords was similar in both dietary groups ($P>0.05$). Furthermore, the estimated total number of OCT4, DAZL, and VASA-positive cells in the whole fetal ovary were unaffected by maternal diet ($P>0.05$). OCT4-positive cells were more abundant than DAZL ($P<0.01$), which was 2-fold more abundant than VASA-positive cells ($P<0.001$), suggesting germ cell development was occurring at this time. However, the ratios between different germ cell markers was the same across the different diets ($P>0.05$). In conclusion, there is no evidence that on day 65 of gestation the gross fetal ovarian structure or number of germ cells were influenced by dietary protein restriction in sheep.

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O026

Fetal androgens determine adult pancreatic function

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Introduction
Maternal androgen excess in pregnant sheep programmes a PCOS-like phenotype in female offspring. We previously demonstrated a hyperinsulinaemic offspring phenotype in response to maternal androgen excess, but such regimens also increase estradiol concentrations, and may effect adrenal steroidogenesis, hence the role of androgens remains unclear.
Materials and Methods
To examine contributions of different steroid classes, and to determine if adult hyperinsulinaemia is a direct consequence of altered pancreatic development during fetal life, we applied steroids directly to ovine fetuses at d62 and d82 of gestation, and examined fetal (d90) and postnatal (11 months old) pancreatic structure and function. Alpha and beta cell content was determined by immunohistochemistry, insulin secretion by ELISA, and insulin signalling by QPCR and Western blotting.
Results
Of three classes (androgen – Testosterone propionate (TP), estrogen – Diethystilbesterol (DES) and glucocorticoid – Dexamethasone (DEX)) of steroid agonists applied, only androgens (TP) altered pancreatic development. Beta cell numbers were significantly elevated in prenatally androgenised female fetuses ($P=0.03$) (to approximately the higher numbers found in male fetuses), whereas alpha cell counts were unaffected, precipitating decreased alpha:beta ratios in the fetal pancreas ($P=0.001$), sustained into adolescence ($P=0.0004$). In adolescence basal insulin secretion was significantly higher in female offspring from androgen-excess pregnancies ($P=0.045$), and a hyperinsulinaemic response to glucose challenge ($P=0.0007$) observed. Postnatal insulin secretion correlated with beta cell numbers ($P=0.03$). No alterations in insulin signalling components were evident.
Discussion and Conclusions
Male and female pancreatic structure differs during fetal life, likely due to androgen concentrations. Androgenic stimulation during development gives rise to female postnatal offspring whose pancreas secreted excess insulin due to excess beta cells in the presence of a normal number of alpha cells. We identify that these animal models of PCOS have primary hyperinsulinaemia prior to any insulin resistance-driven compensatory hyperinsulinaemia.

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O027

Androgen causes whitening of brown adipose tissue: Implications for PCOS

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Polycystic ovary syndrome (PCOS) is a common endocrinopathy that is associated with hyperandrogenism- and an adverse metabolic profile including obesity and insulin resistance. Women with PCOS and raised androgen levels exhibit reduced postprandial thermogenesis and this is thought to predispose women with PCOS to weight gain (Robinson et al., 1992, Clin Endocrinol 36: 537–543). Brown adipose tissue (BAT) is important in the dissipation of energy in the form of heat and changes in BAT could explain the reduction of postprandial thermogenesis found in women with PCOS. In this study, we investigated the effect of androgen on the differentiation of BAT, as well as, expression of BAT and mitochondrial genes. Mouse brown preadipocytes were differentiated for 7 days in the presence or absence of the potent androgen dihydrotestosterone (DHT, 10 nM to 10 μM). Our results show that androgen inhibits brown adipose

differentiation in a dose dependent manner. We then treated explants of mouse interscapular BAT with either 100 nM DHT or vehicle for 24 hours. Androgen treatment resulted in reduced expression of several key BAT genes, including UCP1 ($P<0.05$), PGC-1 ($P<0.05$) and Cidea ($P<0.05$). In contrast, genes involved in mitochondrial function were unaffected by androgen treatment. In light of this we have begun to investigate the expression of brown adipose tissue genes invisceral and subcutaneous adipose tissue from women with and without PCOS. Our results show that genes such as UCP1, PGC1 and β3-AR are differentially expressed between visceral and subcutaneous depots. Furthermore, preliminary expression profiles of women with PCOS are consistent with ex-vivostudies of mouse adipose treated with DHT. Together, these data show that androgen causes a whitening of adipose tissue and could provide a molecular explanation for reduced postprandial thermogenesis and the tendency for obesity in women with PCOS.

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Oral Communications 4: Early Development 2

O028

The effect of decidual stromal cells on immune cells in the first trimester of pregnancy

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Introduction

Decidualisation occurs in the secretory phase of the menstrual cycle, transforming endometrial stromal cells to secretory decidual stromal cells (DSC). Extensive cross-talk occurs between DSC and immune cells. In the first trimester of pregnancy the decidua is rich with maternal leukocytes, made up of ~70% NK cells and ~20% macrophages. The number of NK and macrophage cells increases during decidualisation and into the first trimester of pregnancy. Decidual NK (dNK) cells have a distinctly different phenotype to peripheral blood NK with a unique repertoire of activatory and inhibitory receptors. dNK and macrophages have roles in spiral artery remodelling before and during trophoblast invasion.

Materials and methods

DSC, dNK and macrophages were isolated from tissue obtained from first trimester terminations of pregnancy. DSC were re-decidualised *in vitro* using cAMP and medroxyprogesterone 17-acetate (MPA) conditioned media (CM) was collected after 72 hours. Decidualisation was measured by secretion of IGFBP1, PRL and expression of FOXO1. dNK and macrophages were incubated with DSC CM for 6 hour, media was changed to 10% (v/v) FBS RPMI and cells incubated for a further 12 hours. CM was collected IL-6 and IL-8 secretion via ELISA and cell receptor expression via flow cytometer was analysed.

Results and discussion

Decidualisation of DSC increased secretion of IGFBP1, PRL and FOXO1 ($P<0.05$). DSC CM did not alter dNK receptor expression. DSC CM significantly stimulated dNK secretion of IL-8 and IL-6 ($P<0.05$). DSC CM did not significantly alter macrophage receptor expression or secretion of IL-8 or IL-6. These results indicate that decidualised DSC secreted factors do not affect the receptor expression of decidual NK or macrophage cells. However, DSC activated dNK stimulating them to secrete the chemokines IL-8 and IL-6. These results provide support for the hypothesis that DSC interact and stimulate NK cells in the first trimester of pregnancy.

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O029

The role of integrin AVB6 in the regulation of foetal growth in pigs

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Introduction

Large within-litter variation in pig foetal weight may be associated with placental efficiency. Integrin receptors, including AVB6, are present at the attachment site between the foetal and uterine epithelium and may affect the efficiency of attachment. It is hypothesised that the expression of integrin receptors would differ between tissues supplying small foetuses compared to normal-sized littermates. This study compared the distribution and quantity of integrin AVB6 in placental and endometrial tissues supplying different sized foetuses.

Methods

Placental and endometrial tissues associated with the smallest and a normal-sized foetuses were collected from Large White X Landrace gilts at gestational day (GD) 30, and both sizes from both male and female foetuses at GD 60 and 90 ($n=5$, 7 and 5 respectively). Tissues were fixed in Bouin's and immunohistochemistry for integrin AVB6 to estimate percentage staining was performed.

Results and Discussion

Integrin AVB6 was localised at blood vessels, luminal and glandular epithelium of the endometrium and at blood vessels and the trophectoderm of placenta at GD30, 60 and 90. Integrin AVB6 in the trophectoderm supplying male foetuses was higher than in females at GD30 ($P=0.05$). Integrin AVB6 expression per uterine gland of endometrium was increased from GD30 to GD60 and decreased from GD60 to GD90 ($P<0.01$). Within-females, but not males, integrin AVB6 expression per uterine gland was significantly decreased at GD60 compared to GD90. This temporal change was observed within both the small ($P=0.04$) and normal ($P=0.01$) female categories.

This is the first study showing that integrin AVB6 is present in porcine endometrial tissue, with interesting temporal changes in expression detected. Foetal size does not appear to alter the placental or endometrial expression of integrin AVB6. However, this study has highlighted intriguing differences between tissues supplying foetuses of different sex which warrant further investigation.

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O030

Different mechanism of lipid accumulation in embryoblast and trophoblast cells trigger by lipid excess

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Introduction

Differentiation of the embryoblast and trophoblast cell lineages is an ontogenetic milestone in preimplantation embryo development. Both cell lineages are supplied with nutrients from uterine secretions, reflecting an ultrafiltrate of maternal plasma. We have recently shown that an insulin-dependent diabetes mellitus leads to maternal hyperlipidaemia and to a strong increase in intracellular lipids in trophoblast and, especially, embryoblast cells in rabbit blastocysts (Schindler et al. 2014, Endocrinology 10.1210/en.2013-1760).

Our aim was to determine mechanisms of lipid accumulation in the embryoblast and trophoblast in rabbit blastocysts derived from a diabetic (*in vivo*) and hyperlipidaemic (*in vitro*) environment.

Methods

For *in vivo* analysis blastocysts from diabetic rabbits were flushed from the uterus six days after mating. To evaluate the effect of an increased environmental lipid level in detail, blastocysts from healthy rabbits were cultured *in vitro* with a specified lipid mixture (Gibco, Chemically Defined Lipid Concentrate). Intracellular lipid accumulation was visualised by Oil Red-O staining. Marker molecules involved in lipid metabolism were analysed by qPCR, Western Blot and immunohistochemistry, separately in the embryoblast and trophoblast.

Results and Discussion

In embryoblast cells intracellular lipid droplets and fatty acid uptake (FATP4) and binding (FABP4), as well as PPARg expression were increased after lipid stimulation. In trophoblast cells intracellular lipid droplets were also increased but accompanied by a down-regulation of fatty acid oxidation (CPT1 and PPARa) and fatty acid synthesis (FASN), indicating that two different signalling pathways were activated in blastocysts. Consistently with these results, embryos from diabetic rabbits revealed also an increased expression of FATP4, FABP4 and PPARg in embryoblast cells and down-regulation of CPT1 and FASN in trophoblast cells.

Our study shows that embryoblast and trophoblast handle hyperlipidaemic conditions in different ways, leading in the result to similar phenotypes. This observation underlies the importance of more differentiated analyses in early embryos.

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O031

Ontogeny of molecular transporters in the human placenta

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Introduction
Normal function of the placenta, an essential conduit between mother and fetus, is crucial for a healthy pregnancy. Placental molecular transporters play essential gatekeeping roles, regulating the exchange of nutrients, gases, hormones and diverse molecules between the mother and developing fetus. Expression of some transporters change throughout pregnancy, which may alter fetal sensitivity to maternally-derived nutritional compounds, environmental contaminants (e.g. cigarette smoke chemicals) and medications. We characterised the expression of major molecular transporters in human placentas from late 1st to late 2nd trimester to understand how gestational age and/or fetal sex influence transporter expression.
Methods
Placental and fetal cDNA were genotyped to confirm sex of 48 placentas (8–18 weeks of gestation, male $n=21$, female, $n=27$, MRC/Wellcome Trust Human Developmental Biology Resource [www.hdbr.org]) from electively-terminated normal pregnancies. Transcripts of 49 major transporters were measured by real time qPCR and values normalised against validated house-keeping genes (NormFinder). Linear Regression models were used to analyse gestation- and/or sex-specific differences in placental transporter expression.
Results and Discussion
Transcripts for 31/49 transporters were detectable in the human placenta. No sex-specific expression patterns were observed, but 4 transporters changed with gestational age. The thyroid hormone transporter (SLCO4A1) expression decreased with gestational age reflecting the declining need for maternally-derived thyroid hormone. ABCG2, involved in drug/xenobiotic efflux, was highly expressed in earlier stages suggesting a need for protection during organogenesis. Prostaglandin transporter (SLCO2A1) expression increased, suggesting a role in controlling prostaglandin levels, important in maintaining pregnancy. SLC22A17 also increased, likely supporting nutritional transport and iron homeostasis. Overall, our results suggest that transporters relating to basal metabolic processes, nutrient delivery and drug resistance are stably expressed across gestation maintaining fetal nutrition and protection. Characterising differential transporter expression will improve understanding of critical windows of fetal vulnerability to drugs and toxicants.

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O032

The target organs of human placental micro- and nano-vesicles

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Introduction
The human placenta continuously extrudes, into the maternal circulation, vast quantities of extracellular vesicles (EVs) which have the ability to alter maternal physiology. There are three sizes of placental EVs multinucleated macro-EVs, subcellular micro- and nano- EVs. Macro-EVs are trapped in the maternal lungs due to their large size. We investigated the organs with which micro- and nano-EVs interact in vivo and their potential effects EVs on vascular function.
Methods
Placental micro- and nano- EVs, isolated from cultured human placentae by sequential ultracentrifugation (20,000 g and 100,000 g, respectively), were labelled and injected into groups of 4–6 female CD1 mice via the tail vein. After 2 minutes, 30 minutes or 24 hours, fluorescence in the brain, thymus, lungs, heart, liver, spleen, kidneys was quantified using an IVIS Kinetic Imager at 605/640 nm. Mesenteric resistance artery function was assessed using wire myography. Statistical significance was assessed by the Fisher Exact test and two-way ANOVA.
Results and Discussion
At two minutes post-injection, micro-EVs were detected in the lungs, while nano-EVs were detected in the lungs, liver and kidneys ($P=0.026$). At 30 minutes, the distribution of nano-EVs was unchanged whereas, micro-EVs remained in the lungs but had also spread to the liver and kidneys. By 24 hours, micro-EVs remained only in the liver and kidneys while nano-EVs were cleared from the kidneys but remained in the lungs and liver ($P=0.005$). Myography indicated that there was no effect of nano-EVs on the ability of mesenteric arteries to vasoconstrict or undergo endothelium-dependent and –independent relaxation, in response to U46619, acetylcholine or sodium nitroprusside respectively ($n=5$). We have shown that placental micro- and nano- EVs have distinct patterns of distribution *in vivo* possibly reflecting their different targeting receptors. Preliminary work indicates that nano-EVs from normal placentae do not affect resistance artery function, at least in non-pregnant animals.

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O033

Spermbots: Magnetic microrobots that assist sperm cells on their journey, opening new routes to assisted reproduction

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Introduction
The interdisciplinary field of microrobotics recently sparked interest especially in potential applications of mobile microscale devices that can operate remotely-controlled inside the human body. This study presents an approach towards such an application, namely assisted reproduction with the help of tiny sperm carriers, so-called spermbots. Spermbots are synthetic tubes or helices that couple to single sperm cells and assist their movement by providing guidance or propulsion when actuated by external magnetic fields. Sperm cells with insufficient motility or navigation capabilities are thus supported on their journey, *in vitro* and possibly even *in vivo*, which signifies a wholly new approach to counter male factor infertility.
Methods
Tubular and helical microdevices were fabricated by nanomembrane-roll-up (Magdanz et al 2016 Adv Mater doi: 10.1002/adma.201505487) and direct laser writing (Medina-Sánchez et al 2016 Nano Lett 16: 555–561). The synthetic devices were actuated *in vitro* with weak magnetic fields and their coupling to bovine sperm cells was recorded under the microscope. Imposed hypoosmotic swelling of sperm cells facilitated proper sperm selection. Motion performance and biocompatibility studies with coupled spermbots served to evaluate their capability to emulate potent sperm behavior.
Results and Discussion
Single sperm cells were successfully captured by tubular and helical microdevices while remaining viable and intact. External magnetic guidance of tubular carriers and directed propulsion of helical motors was achieved. Tubular spermbots were able to release captured sperm via thermoresponsive shape alteration helical spermbots released sperms by reversion of their magnetically imposed rotation. With helical spermbots, sperm transport and release at the zona pellucida of an oocyte was shown *in vitro*. Our results illustrate the potential of assisted fertilization with microrobots. This novel approach is meant to deal with severe cases of male factor infertility, with its main strength lying in its potential *in vivo* applicability. (funded by DFG priority program SPP1726).

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Poster Sessions

P001

Abstract withdrawn.

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P002

A retrospective review of outcomes following endometrial scratch procedure in IVF at Fertility Exeter

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Introduction

Mechanical endometrial injury (i.e. endometrial scratch) in the cycle preceding ovarian stimulation for IVF has been proposed to improve implantation in women with unexplained recurrent implantation failure (RIF).

During the implantation window, there is a cross-talk between the embryo and endometrium to allow attachment, adhesion and invasion of the embryo. Endometrial scratch (ES) is suggested to induce changes in the immune system in the uterus which stimulate natural killer cells, which are thought to be a key part of implantation.

Some studies have demonstrated an increase in pregnancy rates among women who have had an ES with a history of RIF. Others have failed to demonstrate benefit. Conclusions regarding the efficacy of the procedure are limited by heterogeneity between studies.

We aimed to assess outcomes in women who have undergone an ES during IVF treatment for RIF at the Fertility Exeter clinic from January 2015–2016.

Methods

A retrospective review of data collected on IVF treatment cycles undertaken at Fertility Exeter from January 2015 to January 2016. Sub-analysis separating frozen embryo transfer cycles and fresh embryo transfer cycles was also performed. Scratches were performed in the preceding treatment cycle according to the clinic protocol.

Results

For completed treatment cycles (i.e. from ovulation induction to embryo transfer) clinical pregnancy rates were 35.1% among those who had an ES, compared to 30.3% who did not undergo ES. In those who had a frozen embryo transfer (FET) clinical pregnancy rates were 54.5% in the ES group, versus 32.3% in those who did not have a scratch. For fresh embryo transfers, clinical pregnancy was achieved in 26.9% of those who did have ES compared to 29.8% of those without ES. The difference between these groups was not found to be statistically significant. There was no difference in the rate of miscarriage between treatment groups.

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P003

Bi-directional regulation of miR-125a-3p expression by mural and cumulus granulosa cells of mice pre-ovulatory follicles

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Introduction

MicroRNAs, transferred between cells through gap-junctions and fluids, serving as inter-cellular signaling molecules. miR-125a-3p, which can be found in follicular-fluid, is expressed and down-regulated by hCG in mural-granulosa-cells. Our preliminary results show that similar effect of hCG in oocytes enables resumption of first-meiotic division. Cumulus-cells and oocytes maintain diverged relationship that includes bi-directional transfer of RNAs and proteins. Because mural-cells transmit the LH/hCG-ovulatory stimulus to the cumulus-oocyte-complex we hypothesized that miR-125a-3p is regulated within the follicle, which serves as a unit enabling synchronization towards ovulation.

Methods

Mural and cumulus-cells were isolated from pre-ovulatory mice follicles 48 hours after PMSG administration, lysed or transfected with scramble-miR or miR-125a-3p-mimic and incubated over-night. Freshly isolated mural/cumulus-cells were seeded and incubated over-night with conditioned medium of transfected-cells. Levels of pri-miR-125a, miR-125a-3p/5p and Fyn-mRNA (downstream target of miR-125a-3p) were measured by real-time PCR.

Results and Discussion

miR-125a-3p expression and activity (indicated by Fyn-mRNA levels) in cumulus-cells cultured in conditioned medium of miR-125a-3p-transfected mural-cells, was higher than in cells cultured in conditioned medium of scramble-transfected cells suggesting that mural-cells modulate expression of miR-125a-3p in cumulus-cells through secretion of a yet unknown factor. We examined whether mural-cells are the lone source of miR-125a-3p or it is transcribed by cumulus-cells. Pri-miR-125a was detected in both cell-types, with higher level in cumulus-cells, similar to the levels of miR-125a-3p/5p. The relative percent of miR-125a-3p/5p from pri-miR-125a was equal in mural and cumulus-cells, suggesting that either they are similarly regulated or that the cells maintain a bi-directional communication enabling them to sense and adjust miR-125a-3p/5p level. Utilizing our cell-model we show that cumulus-cells can also modulate miR-125a-3p expression in mural-cells. (Our results imply that miR-125a-3p is tightly regulated within the follicle. Secretion of miR-125a-3p or of an unknown factor allows mural and cumulus-cells to co-regulate the level of miR-125a-3p expression.)

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P004

The function of Histone Variants in female infertility

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Histone variants can replace canonical histones in the nucleosome and modify chromatin structure and gene expression. The histone variant H3.3 preferentially associates with active chromatin and has been implicated in the regulation of a diverse range of developmental processes. We recently showed that maternal Hira, a chaperone for the histone variant H3.3, is required for mouse development past the zygote stage. Male pronucleus formation is inhibited upon deletion of Hira due to a lack of nucleosome assembly in the sperm genome. Hira mutant oocytes are incapable of developing parthenogenetically, indicative of a role for Hira in the female genome. Our results demonstrate that Hira-mediated H3.3 incorporation is essential for parental genome reprogramming and reveal an unexpected role for rRNA transcription in the mouse zygote. In addition, we also reported that the specific knockdown of H3.3 in fertilized mouse zygotes leads to developmental arrest at the morula stage. Loss of H3.3 leads to over-condensation and mis-segregation of chromosomes as early as the two-cell stage, with corresponding high levels of aneuploidy. H3.3-deficient embryos have significantly reduced levels of markers of open chromatin, such as H3K36me2 and H4K16Ac. In addition, H3.3 KD embryos have increased incorporation of linker H1. These results reveal that H3.3 mediates a balance between open and condensed chromatin that is crucial for the fidelity of chromosome segregation during early mouse development. In summary, Hira-mediated H3.3 incorporation is essential for mouse early embryo development. Further investigation will focus on dissection of its roles in female infertility and the mechanisms of ribosomal RNA transcription.

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P005

Abstract withdrawn.

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P006

Does Neuromedin B exert a local modulatory effect on ovarian steroidogenesis or cell proliferation?

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Introduction
Neuromedin B (NMB), a highly conserved bombesin-related decapeptide originally isolated from porcine spinal cord, has various physiological effects including the regulation of exocrine and endocrine secretions. The current aims were to investigate whether: (1) NMB is expressed in the bovine ovary (2) NMB or NMB antagonist can modulate ovarian steroidogenesis or proliferation by cultured bovine theca (TC) and granulosa (GC) cells.
Methods
GC ($n=40$) and TC ($n=44$) samples retrieved from bovine antral follicles (2–18 mm) were categorized into five size classes. Early, mid and regressing corpora lutea (CL) were also collected ($n=17$). Total RNA was harvested for qPCR analysis and data were analysed using the $\Delta\Delta C_T$ method using β-actin for normalization. Bovine TC and GC cultured under both non-luteinized (LH or FSH) and luteinized (forskolin) conditions were treated for 5 days with NMB (10^{-10}–10^{-6}M), NMB antagonist (BIM 23042 10^{-10}–10^{-6}M) or a combination of the two. Steroid secretion (androstenedione, oestradiol, progesterone) was measured by ELISA and viable cell number determined by neutral red uptake assay. Results are based on 3–8 independent cultures.
Results and Discussion
Two-way ANOVA showed a significant effect of follicle cell-type ($P<0.01$) and cell-type x follicle category interaction ($P<0.05$) with NMB expression declining in GC whilst increasing in TC during follicle development. NMB expression also varied according to CL stage ($P<0.05$). However, TC/GC culture experiments using NMB or its antagonist offered no evidence that NMB has a direct intra-ovarian role to modulate basal or LH-induced TC androgen production, basal or FSH-induced GC oestrogen production or basal or forskolin-induced progesterone production by luteinized TC/GC. However, NMB dose-dependently increased viable cell number by non-luteinized GC ($\sim$ 2.3-fold $P<0.05$) without affecting TC or luteinized TC/GC number. Further experiments are in progress to ascertain whether NMB enhances GC proliferation or reduces cell death.

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P007

Alterations in the vasculature of placental but not endometrial tissue associated with small porcine foetuses compared to their normal-sized littermates

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Introduction
Low piglet birth weight has severe consequences for neonatal and adult development that cannot be remedied post-natally. It is hypothesised that impaired foetal growth occurs due to inadequate placental vascularisation.
Methods
Endometrial and placental tissues supplying small and normal-sized foetuses were collected from Large White x Landrace gilts at gestational days (GD) 30 ($n=5$), 60 ($n=7$) and 90 ($n=5$) for immunohistochemical staining of the endothelial cell marker, CD31.
Percentage staining (PS) of the chorioallantoic membrane (CAM) was determined on GD 60 and 90. GD60 placental stromal analyses included quantification of PS, number of blood vessels (BV) and internal and external blood vessel diameters. The number of BVs, glands and PS were quantified in endometrial samples (GD 30 and 60).
Results and Discussion
PS was increased in the GD60 CAM associated with small foetuses (mean$\pm$ SEM; 4.58$\pm$1.13%) compared to those supplying normal-sized foetuses (2.17$\pm$ 0.37%; FPr$=$0.037). This difference was also significant when comparing small and normal-sized males ($P\leq0.05$), but not females. No differences in CAM PS at GD90 were detected.
Stromal PS at GD60 was increased (FPr$=$0.010) in placentas supplying small foetuses (0.61$\pm$0.12%) compared to their normal littermates (0.30$\pm$ 0.04%). No differences in the number of BV present in the placental stroma (GD60) were detected. Internal ($P=0.029$) and external ($P=0.085$) BV diameters were increased in placentas associated with small males compared to normal-sized males.
A decrease in the number of glands (FPr$<$0.001) and blood vessels (FPr$<$0.001) present in the endometrial samples was observed between GD 30 and 60. No differences were detected in any other parameters investigated in the endometrium.
These findings suggest that the vasculature of placentas supplying small foetuses is altered at GD 60 and 90. Small males have the most striking alterations, highlighting the presence of a potential compensatory mechanism.
(Funding: BBSRC and Edinburgh University).

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P008

Generation of GFP transgenic cynomolgus monkeys

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Introduction
Nonhuman primates are considerered valuable human disease models. There are not many reports about transgenic monkey and especially no report about GFP cynomolgus monkey. Here, we report for the first time generation of whole body GFP expressing cynomolgus monkey.
Methods
We constructed a lentiviral vector carries GFP under control CAG promoter. To generate a transgenic cynomolgus monkey expressed GFP uniformly, we compared injection timing of lentiviral vector. Lentivirus were injected perivitelline space of oocytes befor 4 h of fertilization via intracytoplasmic sperm injection (ICSI) (PreI)or 24 h after fertilization via ICSI (PostI).
Results and Discussion
Transfer of five PostI embryos into three recipients resulted in two pregnancies, one of which ended in miscarriage of twins on 92 of gestation. Although one of the twins (#1) showed no detectable fluorescence, the other (#2) showed strong fluorescence in the face, skin, placenta and brain. Partial GFP expression in the peripheral blood and fibroblast established from tail were observed. An offspring (named PreI Tg #1) from the PreI embryo was born successfully and showed strong fluorescence in the face and placenta. Amnion of PreI Tg #1 monkey showed the uniform GFP expression at the cellular level. Another offspring (named PreI Tg #2) was born, but died 3 days after birth. Uniform GFP expression in the peripheral blood and fibroblast established from tail were observed. Collectively, these results demonstrated that the GFP cynomolgus monkey created by PreI technique expressed GFP uniformly.

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P009

Involvement of MyD88 in B-cell mediated immune response in a mouse model of LPS-induced fetal death

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Introduction
During pregnancy, the maternal immune system has to tolerate the semi-allogenic fetus while protecting the mother and the unborn against pathogens. Subclinical infections that danger the fetus constitute a challenge for the medical system. A useful model to study mechanisms underlying infection-driven fetal death is a LPS-based mouse model. The recognition of LPS, a component of gram-negative bacteria membrane, leads to an immune answer that takes place through TLR4 signaling. In this pathway, MyD88 plays an important role. Having recently shown that IL-10 secreting B cells are important components of the maternal immune response, we now aim to investigate the participation of MyD88 in B cell-mediated fetal protection.
Methods
B cell specific MyD88 knockout mice were generated by mating $CD19^{cre.in}$ and $MyD88^{flox/flox}$ animals. Following female animals were included in our study: wildtype (WT), $CD19^{cre/wt}/MyD88^{flox/flox}$ with MyD88 deficiency in B cells (Het/KO) and $CD19^{cre/cre}/MyD88^{flox/flox}$ lacking B cells and MyD88 (KO/KO). After successful pairing with BALB/c males, LPS (0.6 μl/animal) or PBS was injected at day 10 of the pregnancy. 24 hours later the animals are sacrificed, fetal death rate was determined and tissues were harvested for histological examinations.

Results and Discussion
Animals with B cell specific MyD88 deficiency had an increased fetal death rate compared to all other groups. It seems that the absence of MyD88 in B cells leads to the lethal effect of LPS, while total B cell absence leads only to a mild impact of LPS on fetal survival. Furthermore these animals had thicker spiral arteries compared to the PBS-Group. Ongoing studies will help understanding whether the absence of MyD88 in B cells hinders the secretion of IL-10 and by doing so, dangers fetal survival. Our data sheds light upon novel mechanisms of fetal protection that are worth to be further studied.

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P010

Role of micro-RNAs in thrombospondin-1 expression in bovine luteal cells

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Introduction
FGF2 and thrombospondin-1 (THBS1) expression in corpus luteum (CL) exhibited the most divergent profile of induction by prostaglandin F2a (PGF2α). FGF2, a potent angiogenic pro-survival factor was increased in the Day 4 CL. In contrast, the anti-angiogenic, apoptotic factors, THBS1, transforming growth factor beta 1 (TGFB1) and plasminogen activator inhibitor-1 (PAI-1) were upregulated specifically on Day 11, PGF2α-responsive CL. Functionally, THBS1 reversed FGF2 actions in luteal cells by inhibiting their proliferation, migration, and survival. Furthermore, the expression of THBS1 was suppressed by FGF2 on the contrary, TGFB1 elevated its gene expression. The mechanisms regulating THBS1 expression are not yet understood. microRNAs (miRNAs) represent a possible regulatory mechanism. We therefore aimed to identify relevant miRNA targeting THBS1 expression in luteal cells.
Methods
The TargetScan prediction tool was used to identify candidate miRNAs. Five miRNAs conserved in vertebrates were chosen for further investigation (miR-1, miR-18a, miR-144, miR-194 and miR-221). Luteal endothelial cells (LEC) were transfected with miRNA mimics, then mRNA and miRNA levels were determined by quantitative-PCR. Cell viability were estimated with XTT kit.
Results and discussion
Overexpression of miR-1, miR-194 and miR-221 significantly decreased THBS1 to levels 60–70% lower than in the negative control. All these three miRNAs were endogenously expressed in CL, granulosa cells and LEC, with miR-221 being the most highly expressed. miR-221 was also the only one to be regulated by FGF2 and TGFB1, and in an opposite manner. FGF2 rapidly (after 2 h) upregulated miR-221, before inhibition of THBS1 was detected. Consistent with THBS1 inhibition, miR-221 significantly elevated viable LEC numbers by 160%. TGFB1, simultaneously increased THBS1 and reduced miR-221. Notably, PAI-1, a known TGFB1-induced protein was also reduced by miR-221. These finding suggest that miR-221 inhibits THBS1 in physiologically significant manner. The *in vivo* regulation of miR-221 in relationship to PGF2α remains to be determined.

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P011

Effect of lipopolysaccharide on steroidogenesis and cell migration in the bovine ovary

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Introduction
Lipopolysaccharide (LPS) is a pathogen-associated molecular pattern (PAMP), expressed by gram-negative bacteria. The specific receptor for LPS on host cells is TLR4, one of 11 members of the TLR family involved in innate immunity. Here we investigated i) changes in GC/TC expression of TLR4 during follicle development, ii) The effect of LPS on oestradiol secretion from non-luteinised granulosa cells (GC) and androstendione from non-luteinised theca cells (TC), iii) whether LPS exerts its effect via TLR4 and iv) the effect of LPS on theca and stromal cell (SC) migration.
Methods
GC and TC were isolated from bovine antral follicles (1–18 mm) and RNA extracts used for RT-qPCR analysis of relative gene expression (normalized to β-actin). GC and TC isolated from 4 to 6 mm follicles were cultured (serum-free) for four days with/without LPS and TLR4 inhibitor in the presence/absence of FSH (GC) or LH (TC). Media were assayed for steroids by ELISA and cell-lysates used for RT-qPCR. For wound-healing assays, TC and SC were cultured in 10% serum, a 'scratch' made in the near-confluent monolayer and fresh media added with/without LPS. Cell migration (% wound closure) was assessed over 24 h by time-lapse microscopy.
Results and discussion
Both cell-type and follicle category affected ($P<0.001$) levels of TLR4 mRNA during follicle development. Expression increased with follicle size in both cell types. LPS suppressed FSH-induced estradiol secretion by GC ($P<0.01$) and LH-induced androstendione secretion by TC ($P<0.01$). In GC, LPS down-regulated ($P<0.001$) CYP19A1 and up-regulated TNF-α and GPR77 expression. In TC, LPS down-regulated CYP17, INSL3 and StAR ($P<0.001$) while upregulating TNFR1 and NFkB ($P<0.001$). The inhibitory effect of LPS on GC/TC steroid secretion was blocked by TLR4 inhibitor. However, LPS had no significant effect on either TC or SC migration. Results confirm a profound inhibitory action of LPS on follicular steroidogenesis but it did not affect cell migration.

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P012

Investigation of the relationship between HA binding and sperm function tests including sperm DNA damage and chromatin maturity

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Introduction
During spermiogenesis, which is the last stage in spermatozoa maturation, a spermatozoa plasma membrane remodelling stage occurs which promotes the up-regulation of membrane receptors to aid the zona pellucida binding e.g. hyaluronic acid (HA) receptors.
Researches have demonstrated that spermatozoa that are able to bind to HA *in vitro* are, in fact, more mature spermatozoa that have completed specific maturation processes and are ultimately more likely to reach the oocyte and fertilise it. These maturation processes include plasma membrane remodelling, cytoplasmic extrusion and finally, nuclear maturation of the spermatozoa. It has been noted that spermatozoa selected using HA as a selector exhibit characteristics such as minimal DNA fragmentation, normal morphology, and lower frequency of chromosomal aneuploidies, ultimately supporting the use of the HA binding assay as a sperm selection method for ARTs.
In the current project the ability of good quality (recovered from 90% density gradient or pelleted) and bad quality spermatozoa (recovered from 45% density gradient or interphase) in binding to HA was tested. We also compared the levels of DNA damage and chromatin maturity in pelleted and interphase and also in HA-bound and unbound spermatozoa.
Methods
The HBA® slides (Origio) were used to check the ability of binding pelleted and interphase spermatozoa to HA.
After separation of human spermatozoa using 45–90% density gradient centrifugation (DGC), spermatozoal suspension (pelleted and interphase spermatozoa) was placed onto the assay chamber and the chamber was incubated at RT. As a result, spermatozoa which have HA receptors are able to bind to the HA-coated slide with an active beating tail.
The mean percentage (s.d.) of HA-bound spermatozoa was calculated (for both the interphase and pelleted spermatozoa) for 15 different human samples.
The levels of DNA damage and chromatin maturity was checked using acridine orange (AO) and aniline blue (AB) staining in pelleted and interphase and also in HA-bound and unbound spermatozoa.
Results and discussion
The results showed that there is a significant difference ($P<0.0001$) between the mean percentage (s.d.) of HA-bound spermatozoa in pelleted compared to the interphase spermatozoa.
Our results also confirmed that pelleted spermatozoa had significantly lower levels of DNA damage ($P<0.0001$) and higher levels of chromatin maturity ($P<0.05$) compared to interphase spermatozoa.
In addition we illustrated higher levels of chromatin maturity and lower levels of DNA damage in HA-bound spermatozoa (separated using a HA-coated surface) compared to HA-unbound spermatozoa ($P<0.0001$).
In conclusion our results confirmed that HA binding assay is a good method for selection of mature spermatozoa.

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P013

Characterisation of extracellular vesicles produced by the Porcine oviductal epithelial cells using size exclusion chromatography

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Introduction
The interaction of gametes and embryo with the maternal environment has a crucial impact on gametes maturation, embryonic development and subsequent pregnancy success. Recent studies have recognised extracellular vesicles (EVs) as a potent vehicles for intercellular communication. Defining the type of EVs which are produced by different reproductive cells will help us to understand how these structures can influence reproductive processes. The aim of the current investigation is to characterise EVs secreted by Porcine oviductal epithelial cells (POECs) *in vitro* in conditioned medium (CM).
Methods
EVs were purified from CM by size exclusion chromatography (SEC) using Sepharose CL-2B SEC columns. Twenty eluted fractions each 500 µl were collected and analysed by Zetaview nanoparticle tracking analyser (Particle Metrix GmbH, Meerbusch, Germany) to measure EVs concentration and size distribution. Bicinchoninic acid protein assay was used to determine the efficacy of SEC in separating vesicles from soluble CM proteins. One-dimensional gel electrophoresis was used to visualize the protein profile of the purified EVs. Collected fractions were investigated for the presence of tetraspanin CD63 protein (cell surface marker for EVs) using western blot analysis.
Results and discussion
Size particle analysis confirmed the presence of particles of 70–150 nm with the concentration 1×10^6 to 1×10^7 particles/ml from fraction 4 to 12. Negligible soluble protein was detected until fraction 12. The bulk of protein started to elute from fraction 13 onwards. The presence of CD63 was detected in SEC fraction 4 to fraction 12. In conclusions, SEC methodology efficiently isolated EVs from POEC CM with low levels of soluble proteomic contaminants. The obtained data will enhance our knowledge of periconception environment and the early stages of communication between maternal tract, gametes and embryo.

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P014

PI3K/AKT signaling pathway involvement in motility loss associated with prohibitin downregulation in sperm from infertile men

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Introduction
Phosphoinositide 3-kinase (PI3K) activity has been reported to be critical to sperm motility and mitochondrial ROS generation while mitochondrial membrane protein Prohibitin (PHB) controls PI3K/AKT pathway in somatic cells by regulating mitochondrial function. Our recent findings showed that sperm PHB expression is significantly decreased in infertile men with poor sperm quality by regulating mitochondrial morphology and function. The objective of this study is to test if PHB expression in sperm is associated with the PI3K/AKT pathway.
Methods
Semen samples from 101 male subjects between 30 and 40 years old attempting ICSI/IVF were collected and then assayed by semen analysis according to 2010 WHO standards. Contaminating leucocytes are removed from all samples by using magnetic Dynabeads coated with a monoclonal antibody against CD45 and confirmed using a zymosan provocation assay. After then, the level of phosphorylation of PI3K/AKT pathway in sperm was detected using SDS–PAGE and Western blot in infertile men with poor sperm motility (asthenospermia, A) and/or low sperm concentrations (oligoasthenospermia, OA).
Results and discussion
Our results demonstrate a significantly lower expression of P110 catalytic subunit but a higher P85 regulatory subunit of PI3K in sperm from A and OA subjects than that from normospermic (N) subjects. However, the findings of significantly lower level of phosphorylation of P85 regulatory subunit of PI3K may result from the significantly lower level of phosphorylation of PTEN in sperm from A and OA subjects. Consequently, the significantly downregulated phosphorylation of AKT (pSer^{473} and pThr^{308}) shown in sperm from A and OA subjects, may result from dephosphorylation in sperm with a higher level of mitochondrial ROS as reported previously. Collectively, our observations suggest that downregulated PI3K/AKT pathway shown in infertile men with poor sperm motility may be associated with decreased PHB expression. (This project was supported by National Natural Science Foundation of China, grant number 81270738).

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P015

Effect of atrazine on sperm mitochondrial function, acrosome reaction and fertilization competence: The bovine model

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Introduction
Atrazine (ATZ) is one of the most extensively used herbicides, known as a ubiquitous environmental contaminant and found in water sources. ATZ was detected in human amniotic fluid, serum and urine, however, the risk associated with ATZ exposure on sperm is less known.
Methods
Sperm was isolated from fresh ejaculates or testicular-epididymis compartments (head, body, tail) and capacitated *in vitro* for 4 h with 0, 0.1, 1 or 10 µM ATZ. The integrity of plasma- and acrosome membranes and mitochondrial membrane potential ($\Delta\Psi$m) were examined simultaneously by fluorescent staining at 0, 2 and 4 h of incubation. After capacitation, acrosome reaction (AR) was induced by Ca^{2+} ionophore and the proportion of sperm underwent pseudo- or induced-AR was determined. In addition, oocytes ($n=50$–70 per group 3 replicates) were aspirated from ovaries, *in-vitro* matured (22 h) and fertilized (18 h) with sperm capacitated with 0.1 or 1 µM ATZ. Cleavage and blastocyst formation rates were evaluated after 42 h and 7 days post-fertilization, respectively.
Results and discussion
ATZ had a prominent effect on sperm isolated from the epididymis tail, expressed by disruption of membrane integrity, mostly at low concentrations. Both pseudo- and induced AR were impaired when sperm isolated from ejaculate or epididymis tail were incubated with ATZ (0.1 µM $P<0.05$). $\Delta\Psi$m was affected by ATZ (1 µM $P<0.0009$) only in the later. Pre-fertilization exposure of sperm to 1 µM ATZ resulted in a lower proportion of embryos that cleaved in to 2–4 cell-stage ($P<0.005$) or developed to blastocyst ($P<0.02$). The findings explore the harmful effect of ATZ on sperm viability, acrosome integrity and mitochondrial function. These were associated with reduced fertilization competence and blastocyst formation, indicating the risk associated with ATZ exposure even at low ecologically relevant doses and for short time.

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P016

Effect of pre-*in vitro* maturation using PACAP on nuclear and cytoplasmic maturation in porcine cumulus-oocyte complexes derived from small follicle

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For developmental competence of oocyte derived from small follicle (~3 mm in diameter SF), pre-IVM system was developed for *in vitro* culturing SF. The purpose of this study is to establish the optimal phase and concentration for exogenous addition of pituitary adenylate cyclase-activating peptide (PACAP) on pre-IVM. To establish the appropriate phase for pre-IVM, we assessed nuclear status according to culture duration. The result of the nuclear stage assessment of the COCs (cumulus oocyte complex) from SF are as follow: metaphase I (MI) stage of 0 h (0%), 6 h (0.5%), 12 h (4.8%), 18 h (9.6%) and 24 h (13.4%). The rate of germinal vesicle breakdown (GVBD) and germinal vesicle (GV) in groups between 12 h and 18h groups was no statistically significant difference. Nevertheless, the result of MI stage compared with 0 h and 6 h group showed that the 18 h group accelerated significantly meiotic resumption ($P<0.05$). PACAP was treated on pre-IVM according to concentration. After 18 h, The 10 µM group showed a significantly ($P<0.05$) the highest rate on meiotic arrest of COCs: GV stage of control (60.5%), 500 fM (64.%), 1 nM (74.4%), 100 nM (69.9%) and 10 µM (82.1%). COCs obtained from follicles 4–6 mm in diameter

(MF control) and SF and subjected to IVM for 42 h. In the pre-IVM group, COCs obtained from SF and matured with non treatment (Pre-SF(−)PACAP) and 10 μM PACAP (Pre-SF(+)PACAP) for 60 h. After IVM, Pre-SF(+)PACAP group (91.7%) showed significantly increased nuclear maturation than control and Pre-SF(−)PACAP group (81.7% and 81.7%) ($P<0.05$). The Pre-SF(+)PACAP group showed significantly ($P<0.05$) increased GSH levels compared with SF and Pre-SF(−)PACAP groups. These results indicated that PACAP is able to delay oocyte meiotic maturation during pre-IVM and consequently improve nuclear and cytoplasmic maturation after IVM. This work was supported by Korea Institute of Planning and Evaluation for Technology in Food, Agriculture, Forestry and Fisheries (IPET) through Advanced Production Technology Development Program, funded by Ministry of Agriculture, Food and Rural Affairs (MAFRA) (grant number: 115103-02), Republic of Korea.

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P017

Treatment of GDF8 during *in vitro* maturation increased phosphorylated SMAD2/3 and improved *in vitro* fertilized embryo developmental competence

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The purpose of this study is the effects of growth differentiation factor 8 (GDF8) and SB431542 (SB) on porcine oocytes *in vitro* maturation (IVM), subsequent embryonic development after in vitrofertilization (IVF). We were investigated the effect of GDF8 and SB treatment during IVM on nuclear maturation, intracellular glutathione (GSH), reactive oxygen species (ROS) levels, analyzed specific gene transcription and translation levels in cumulus cells after IVM, and embryonic development and transcription pattern after IVF. Data were analyzed by on way ANOVA. The 1.318 ng/ml of GDF8 and 5 ng/ml of SB were added during IVM followed experiment design as control, SB, SB+GDF8, and GDF8 treatment groups. After 44 h of IVM, GDF8 group (90.4%) showed significantly increased nuclear maturation than other groups (85.4, 78.9, 85.4 and 90.4%, respectively), and SB group showed significantly lower maturation than control ($P<0.05$). The GDF8 group showed significantly ($P<0.05$) decreased intracellular ROS and increased GSH levels compared with other groups. SB+GBF8 group showed significantly better cytoplasmic maturation than SB group. The GDF8 group showed highly increased PCNA and Nrf2 and cumulus expansion factors COX-2, Has2, Ptx3 and TNFAIP6 mRNA transcription level in cumulus cells after IVM. In protein expression level, GDF8 group showed significantly increased phosphorylated SMAD 2/3 per SMAD 2/3 ratio than control ($P<0.05$). In IVF embryonic development, GDF8 group showed a significantly ($P<0.05$) higher blastocyst total cell number compared with control and SB groups (87.2, 93.9, and 119.4 respectively). The gene expression level showed in IVF BL, the developmental competence marker PCNAand POU5F1 transcription levels were tended to increase in GDF8 group compared with control ($0.05<P<0.1$). In conclusion, treatment of GDF8 during IVM significantly improved the IVF embryo developmental competence and effected on transcription pattern, and redeemed developmental potential from SB inhibition by increasing P-SMAD2/3 level.

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P018

Establish of a transgenic neural cell line with an astrocyte-specific inducible CreERT2 system

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Transgenic (TG) pigs are currently regarded as an important animal model for various biomedical researches including a disease modeling and a regenerative medicine. We tried to develop a pig astrocyte-specific CreER^{T2}-LoxP recombination system as a part of TG pig model of brain tumor arisen from astrocytic cell lineage. We designed two vector systems one retains pig glial fibrillary acidic protein (GFAP) promoter-CreER^{T2} transgene and the other has GFP gene flanked by LoxP sites which can be eliminated through the CreER^{T2}-mediated recombination. Then, generated TG pigs through somatic cell nuclear transfer (SCNT) technique were analyzed whether the GFAP-CreER^{T2}-LoxP recombination had been occurred. SCNT and embryo transfer were performed three times to just before ovulation state of the surrogate mothers. One of them was pregnant, and delivered five transgenic piglets to 115 days after pregnancy. Results from gDNA in skin tissues of TG piglets were confirmed that transgenes are introduced. It was confirmed that CreER^{T2} gene expression with the GFAP promoter was highly expressed only in the cerebrum by real-time PCR. After 3 months, we were oral administration of 15 mg/kg of tamoxifen during five days, and then euthanized after 7 days. As a result of PCR, was confirmed that the recombination was induced in the cerebrum. However, the other organ samples did not occur recombination. In addition, the TG neural cell line was established by primary culture of cerebrum and it was confirmed the induction of recombination by PCR. In conclusion, CreERT2 gene, expressed by the GFAP promoter, was found to exist in the specific part of the cerebrum. The observation in the study suggests GFAP-CreER^{T2}-LoxP recombination system consisting of two vector construction works properly and specifically *in vivo* pig brain. This work was supported by the National Research Foundation of Korea Grant funded by the Korean Government (*NRF-2013R1A2A2A04008751*), Republic of Korea.

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P019

Effect of lysophosphatidic acid (LPA) on *in vitro* maturation of porcine oocytes and subsequent embryonic development after parthenogensis and *in vitro* fertilization

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Introduction
Lysophosphatidic acid (LPA) is a signaling molecule derived from phospholipid known to have biological activities such as stimulating proliferation and differentiation. The purpose of this study was to investigate the effect of LPA on IVM of porcine oocytes and subsequent embryonic development after IVF and parthenogenetic activation (PA).
Methods
We examined nuclear maturation, intracellular GSH and ROS levels and subsequent embryonic development after IVF and PA in porcine oocytes matured in 0.1% PVA-TCM199 containing either 0, 10, 30, or 60 μM LPA.
Results and discussion
After 44 h of IVM, the 30 μM LPA group showed significant ($P<0.05$) increased in nuclear maturation (90.31%) compared other groups (82.50, 86.22 and 86.72%, respectively). The 30 μM LPA group exhibited a significant increase in intracellular GSH levels and decrease in intracellular ROS levels compared with other groups. Oocytes matured with 30 μM LPA during IVM had significantly higher cleavage rates after PA (81.51%) than other groups (74.19, 77.53 and 74.63%, respectively). The blastocyst formation rates, also, 30 μM LPA group showed significantly higher (62.04%) than other groups (50.03, 55.02 and 57.09%, respectively). The IVF embryonic competence, 30 μM LPA group had significantly higher cleavage rates (70.58%) than other LPA groups (63.83, 67.76 and 67.07%, respectively). Likewise, the blastocyst formation rates showed significantly higher in 30 μM LPA group (37.87%) than other groups (31.07, 33.93 and 33.97%, respectively). The expression of anti-apoptotic genes in cumulus cells (caspase-3) was decreased, significantly. In conclusion, the treatment with 30 μM LPA during IVM improved the developmental potential of PA and IVF embryos by increasing the GSH level, thereby decreasing the ROS level during oocyte maturation. In addition, LPA was shown to affect the anti-apoptotic during the maturation of Oocytes in cumulus cells.

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P020

Effects of the chemotherapy drugs cisplatin and doxorubicin on the follicles of the human ovary

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Introduction
The effect of chemotherapy treatment on premenopausal women's fertility has long been of concern. Here, we investigate the direct actions of chemotherapy drugs on the human ovary, examining effects of two commonly used drugs, cisplatin and doxorubicin.
Method
Ovarian cortical tissue samples were collected from patients aged 27–34 who were undergoing elective caesarean section, with informed consent and Ethical Committee approval. Tissue was cut into small (0.5 mm×1 mm×1 mm) fragments and each piece placed on floating polycarbonate membranes in McCoy's culture medium with supplements. After 24 h, cisplatin or doxorubicin were added at single-dose (5 and 1 μg/ml respectively) or double-dose (10 and 2 μg/ml respectively), or with a combination of single-doses of both drugs. Twenty-four hours later, all ovaries were moved to drug-free medium for 96 h, with bromodeoxyuridine (BrdU) added during the final 24 h of culture. Follicle number and health status was assessed histologically and levels of stroma cell proliferation (immunohistochemistry for BrdU, Abcam, Ab6326) and apoptosis (cleaved caspase-3, Cell signalling Technology, 9661S) examined.
Results and discussion
Double-doses of either cisplatin or doxorubicin resulted in a significantly greater percentage of unhealthy follicles, rising from around 30% to 60–80% of all follicles ($P<0.05$ for both), and found specifically at the primary/secondary follicle stage ($P<0.05$ for both). There was no effect of single-dose of either drug. Importantly, the combination treatment, when both drugs were administered together at single-dose, also had no significant effect on follicle health. Cleaved caspase 3 expression significantly increased in the double-dose doxorubicin group and in the combination group ($P<0.05$ for both), while BrdU expression decreased markedly in all treatment groups apart from single-dose cisplatin ($P<0.05$). As such, the most marked effect of treatment was on proliferation rates, which decreased in response to the single-dose of doxorubicin, double-dose of either drug and to the combination treatment.

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P021

Impact of a contrasted metabolism on endometrial and peripheral signalling pathways at implantation in dairy cattle

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Intensive selection for milk production has led to a reduced reproduction efficiency of high-producing dairy cattle. The first month of pregnancy is associated to a high rate of pregnancy failures. In addition to embryo losses, pre-conceptional status of pregnant females has been reported to affect progeny performance after birth. This study aimed to investigate the impact of the conceptus on endometrium physiology and on peripheral blood leucocytes (PBL) gene expression patterns in a bovine model of contrasted maternal metabolism. Primiparous Holstein–Friesian dairy cows were dried immediately after parturition (DRY) or milked twice daily (LACT). Between 65 and 75 day post-partum, oestrous cycle was synchronized and a single embryo was transferred to each cyclic female at 7 day post-oestrus (dpo). At 19 dpo, blood samples were collected then after slaughter concept were recovered from pregnant females (DRY, $n=8$ LACT, $n=5$) and endometrial caruncular (CAR) and intercaruncular areas (ICAR) were dissected from the uterine horn ipsilateral to the corpus luteum. Using total RNA and real-time PCR, we analysed the expression of a selection of genes known to be regulated by the presence of the conceptus (PLET1, SOCS6) and by interferon tau (STAT1,RSAD2, SOCS1, SOCS3) or involved in progesterone (FOXL2, SCARA5) prostaglandin (PTGS2) and oxidative stress (CAT, SOD1,SOD2) molecular pathways. No significant impact of the metabolic status was found on gene expression in PBL. Variance analyses revealed a significant impact of the maternal metabolic status on endometrial genes such as FOXL2 ($P<0.002$ DRY/LACT fold-ratio=2.1 in CAR and ICAR) and SOD2 ($P<0.04$ DRY/LACT fold-ratio=0.8 in CAR and ICAR) mRNA expression. Collectively, our findings prompt the need for deciphering the contribution of FOXL2 to endometrial physiology in the context of contrasting metabolic status in dairy cows. Funded by the European X Seventh Framework Programme FP7/2007-2013, grant agreement number 312097 ('FECUND').

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P022

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P023

Analysis of follicle development in a mouse model with increased fertility

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Introduction
The regulation of follicle development is not well understood, despite its importance in determining fertility, but there is evidence that the oocyte plays a key role. The female C1galt1 Mutant mouse has an oocyte-specific deletion of the T-synthase enzyme and as a result cannot synthesise core 1-derived O-glycans. Mutant females exhibit a phenotype of increased fertility and altered follicle development. To investigate the hypothesis that changes in the development of Mutant follicles would manifest as differences in follicle morphology during development, a histological assessment of ovaries from 3-week-old Control and Mutant mice was carried out.
Methods
This study was approved by the Local Ethical Review Panel (University of Oxford). Ovaries were collected from 3-week-old Control and Mutant mice. Ovaries were fixed, embedded, sectioned and every tenth section stained with haematoxylin and imaged. Healthy follicles with a visible nucleus were analysed using ImageJ software. Follicle stage was determined by the number of layers of granulosa cells and antrum area.
Results and discussion
Analysis of follicle counts confirmed that there are more healthy follicles in Mutant ovaries compared to Control ovaries ($P<0.001$) ($n=4$ Control, $n=4$ Mutant). Oocyte diameter and theca area were the same in Control and Mutant follicles of comparable development. However, Mutant follicles with the same number of granulosa cells as Control follicles have a smaller antrum ($P<0.05$) ($n=29$ Control, $n=48$ Mutant). Therefore, follicle morphology is altered in the Mutant during follicle development. These changes are consistent with the previously proposed model that follicle development is delayed in the Mutant compared to Control mice. Furthermore, it is evidence that one or more oocyte-derived proteins possessing core 1-derived O-glycans has a function in regulating the formation of the antrum. This study was partially funded by Nuffield Department of Obstetrics and Gynaecology.

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P024

Therapeutic doses of phosphoramide mustard cause germ cell death in the prepubertal mouse testis

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The past few decades have seen marked improvements in life expectancy following childhood cancer due, in part, to advances in chemotherapy. While these drugs are effective in treating malignant disease, one of the main adverse outcomes can be infertility. This is of particular concern for prepubertal boys, for whom there are not yet any established methods of fertility preservation. The objective of this study was to gain a better understanding of the effects of the widely-used chemotherapy drug, cyclophosphamide, on the prepubertal testis. This study was carried out in a primary tissue culture model. Immature mouse

testes from pnd5 were dissected, cut into fragments, and each fragment was cultured individually on a membrane floating on 1 ml of medium. After 24 h, half of the medium was replaced with medium spiked with phosphoramide mustard (PM), the active metabolite of cyclophosphamide, to give final concentrations within the range of reported patient plasma concentrations (0.01–10 μM). Twenty-four hours after the drug was added, membranes were transferred into fresh, drug-free medium and cultured for a further 48 h. Testis fragments not exposed to PM throughout the culture period were used as a control. Fragments were then fixed, sectioned and analysed using immunofluorescence for expression of testis cell-type-specific and apoptotic markers. Concentrations of 1 and 10 μM PM markedly decreased germ cells, which fell to 10.29 and 7.18% of control levels respectively. Proliferation in the testis and numbers of Sertoli cells and interstitial cell types showed no significant changes. Expression of the apoptotic marker, cleaved caspase 3, only increased after exposure to the highest, 10 μM, concentration. In conclusion, this study found that *in vitro* exposure of the prepubertal mouse testis to concentrations equivalent to mid-high therapeutic concentrations of PM caused significant cell death, with specific and marked loss of germ cells.

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P025

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P026

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P027

Targeting lactate metabolism can be a novel therapeutic for the treatment of endometriosis

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Introduction
Endometriosis is a chronic, hormone-dependent disorder characterized by the establishment and growth of endometrial tissue in extra-uterine sites, typically within the peritoneal cavity and causes debilitating pain. We have shown that peritoneal mesothelial cells recovered from the women with endometriosis have an altered energy metabolism with increased biosynthesis of lactate as a result of increased aerobic glycolysis. We hypothesize that ectopic endometrial tissue may use the excess lactate produced by peritoneal mesothelial cells as an energy source enhancing both their establishment and growth as endometriosis lesions and by targeting lactate metabolism endometriosis may be resolved.
Methods
Eutopic endometrium, endometriotic lesions, peritoneum (from sites distal and adjacent to the endometriosis lesion) and primary human peritoneal mesothelial cells (HPMCs) were collected with informed consent from women with or without endometriosis. Expression of lactate transporters were analysed in tissue biopsies ($n=5$) by qRT-PCR. Lactate secretion from primary HPMCs from women with and without endometriosis was compared *in vitro*. HPMCs and immortalised mesothelial cells (MeT-5a cell line) were treated with compounds that alter the activity of glycolytic enzymes i) PDK1 (dichloroacetate) and ii) LDHA (galloflavin) and their impact on glycolysis was analysed.
Results and discussion
Endometriosis lesions had higher concentrations of MCT1 mRNA ($P<0.01$) compared with adjacent peritoneum while expression of MCT4 was higher in the peritoneum tissue recovered adjacent to lesions ($P<0.01$). HPMCs from women with endometriosis showed an increased lactate secretion *in vitro* ($P<0.05$). Treatment of HPMCs with dichloroacetate and galloflavin both reduced mRNA expression of key glycolytic markers and lactate secretion. These results suggested that endometriosis is associated with a shift in the metabolic activity of mesothelial cells resulting in increased secretion of lactate (‘Warburg-like effect’) and repurposing of anticancer drugs (dichloroacetate, galloflavin) that target lactate metabolism may offer potential as therapeutics for endometriosis.

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P028

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P029

Membrane potential contributes to generation of high amplitude $[Ca^{2+}]_i$ oscillations and sperm behaviour in human spermatozoa

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Ca^{2+} signaling is crucial for regulation of sperm motility and $[Ca^{2+}]_i$ oscillations may underlie ‘switching’ of sperm behaviors in human spermatozoa, which is believed to be important for sperm progression in the female tract. We have investigated the contribution of membrane potential (Vm) and the sperm Ca^{2+} channel CatSper to $[Ca^{2+}]_i$ oscillations induced by progesterone (P4). Manipulation of Vm was performed using the K^+ ionophore valinomycin (VLN-1 μM), alone or with high K^+ media (100 mM) and confirmed by whole-cell patch clamping. $[Ca^{2+}]_i$ signaling was assessed by loading sperm cells with the Ca^{2+}-indicator fluo-4-AM and stimulating them with 3 μM P4. 29.8+4.53% of cells exhibited $[Ca^{2+}]_i$ oscillations after P4 stimulation ($n=7366$ cells). $[Ca^{2+}]_i$ rose first in the flagellum, consistent with activation of CatSper, then spread actively to the head, apparently triggering Ca^{2+} store release. Two oscillations patterns were observed: ‘rapid’ transients resembling the initial progesterone response and slower transients with lower amplitude. Pre- or post-treatment with VLN to ‘clamp’ Vm at −66.4 mV abolished ‘rapid’ $[Ca^{2+}]_i$ oscillations in 95% of cells but ‘slow’ transients were resistant. Both Ca^{2+} oscillations patterns completely recovered upon VLN washout. Both ‘rapid’ and ‘slow’ oscillations were more resistant to depolarized clamp of Vm (−4.6 mV with VLN/100 mM K^+) and recovery of oscillations was slower after VLN/K^+ removal. Treatment with CatSper inhibitor abolished ‘rapid’ and ‘slow’ $[Ca^{2+}]_i$ oscillations and both recovered rapidly when the inhibitor was removed. Tracking of fluo-4-labelled, free-swimming cells showed switching of sperm behaviour during ‘rapid’ $[Ca^{2+}]_i$ oscillations. Our results show that Vm contributes to generation of high amplitude $[Ca^{2+}]_i$ oscillations and sperm behaviour (probably by regulating CatSper) but low amplitude transients may be regulated differently.
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P030

Investigating the role of the membrane receptor ADGRD1 in female fertility

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ADGRD1 is a member of the adhesion G protein-coupled receptors (aGPCRs), a family of membrane proteins that are categorised by the presence of a GAIN (GPCR autoproteolysis-inducing) domain between a large N-terminal extracellular region that is thought to bind signal-initiating ligands, and the signal-transducing G-protein-coupled seven transmembrane helices. The role and function of many aGPRCs is unclear and most of them are ‘orphan’ receptors, having no identified ligand. A mouse line specifically lacking Adgrd1 has been generated at the Sanger Institute and homozygous females were found to be infertile: no pregnancies were detected, and no pups were delivered. To understand the molecular basis ofAdgrd1 function in female fertility, we are

trying to determine exactly when and where ADGRD1 is expressed and to identify its ligand. Normal-looking eggs are ovulated in response to hormonal stimulation and the number of zygotes recovered after mating is similar in WT and in Adgrd1$^{-/-}$ females. Furthermore, the lack of embryo implantation sites and their absence from the uterus point to an impairment of transport along the oviduct. Ciliary beating and muscle contractions propel embryos towards the uterus, and it is unclear if and how ADGRD1 is involved in any of these processes. If embryos are retained in the oviduct this might lead to ectopic pregnancies. It is estimated that in the UK around one in ninety pregnancies develops into an ectopic one therefore, studying the role of genes like Adgrd1, can help to understand the causes of ectopic pregnancy, and may result in the development of a diagnostic test.

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P031

Effect of pHi on regulation of hyperactivated motility in human spermatozoa

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CatSper channels are expressed specifically in the principal piece of the sperm flagellum. In human sperm Catsper channel activation is polymodal and sensitive to increased pHi, progesterone and other agonists, potentially resulting in synergistic enhancement of Ca^{2+} influx and hyperactivation (1) We have investigated if the interaction of pHi with the CatSper agonist progesterone and 4-aminopyridine (4AP), a potent activator of hyperactivation in human sperm. $[Ca^{2+}]_i$, pHi and motility were monitored using a fluorescent plate reader and CEROS CASA system. Incubation of capacitated human sperm at pH values between 6.0 and 9.0 resulted in proportional changes in pHi. At pHo=7.4 (pHi=6.9) and pHo=8.5 (pHi=7.2) the % hyperactivated cells was 2.50.7 and 12.31.8respectively ($n=21$, $P=2\times10^{-6}$). Treatment with progesterone (0.1–20 μM) to stimulate CatSper channels induced only a modest increase in hyperactivation that was similar at pHo=7.4 and pHo=8.5. The amplitude of this effect was inversely proportional to the spontaneous level of hyperactivation. In contrast, effect of 4-AP (0.2–5 mM) on hyperactivation was significantly greater than progesterone and was stronger and more potent at pHo=8.5 than at pHo=7.4. Resting $[Ca^{2+}]_i$ was higher in cells incubated at pHo=8.5 than at pHo=7.4. Both progesterone (0.001–20 μM) and 4-AP (0.2–5 mM) induced dose-dependent elevation of $[Ca^{2+}]_i$ but at pHo=8.5 the effect of 4-AP was greater than at pHo=7.4. These data show that: i) Catsper activation by progesterone only weakly enhances hyperactivation of human sperm and though alkalinisation increases $[Ca^{2+}]_i$ this treatment does not interact synergistically with progesterone to enhance hyperactivation; ii) the more potent effect of 4-AP on hyperactivation is significantly enhanced at high pHi, suggesting that the action of 4-AP on human sperm $[Ca^{2+}]_i$/hyperactivation involves a different pathway.

Reference

1. Alasmari *et al*. *J. Biol. Chem.* 2013 **288** (9), 6248–6258.

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P032

Gonadotropins induce expression of versican in porcine oocyte-cumulus extracellular matrix and mural granulosa cells

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Introduction

Versican is a large extracellular matrix (ECM) proteoglycan that regulates adhesion, survival, proliferation and migration of the cells, as well as ECM assembly. In rodent follicles, versican V0/V1 expression increases about 4 h after hCG induction, while in bovine granulosa cells no increase was observed post hCG injection, suggesting that versican expression may varies significantly between species. Interestingly, the versican V1 cleaved product G1-DPEAA accumulates in the mouse cumulus matrixin vivo few hours prior ovulation, likely contributing to its expansion. We investigated the spatiotemporal expression of G1-DPEAA cleaved product in porcine follicles during the periovulatory period.

Methods

Porcine oocyte cumulus complexes (OCCs) stimulated in vivo and OCCs and mural granulosa cells (MGCs) stimulated *in vitro* with FSH/LH were undigested or digested with either chondroitinase ABC or *Streptomyces* hyaluronidase. Total, matrix and cell pellet extracts were then analyzed by Western blot by using a versican antibody recognizing the neoepitope DPEAAE.

Results

Both *in vivo* expanded and *in vitro* FSH/LH stimulated porcine OCCs accumulated V1 versican cleaved form (∼70 kDa) in the ECM. Our *in vitro* analysis clearly indicated that the versican ∼70 kDa cleaved product accumulated in the matrix with time, since it was barely visible at 26 h and became quite evident at 44 h of culture. Interestingly, the OCCs samples treated with hyaluronidase showed an additional band of about 75 kDa MW. This ∼75 kDa form was quite evident at 26 h after stimulation, increased slightly at 44 h and was absent in MGCs. Conclusion: V1 versican cleaved form (∼70 kDa) was detected both in *in vivo* and *in vitro* matrix extracts of gonadotropin stimulated OCCs as well as in matrix extracts of MGCs after stimulation with FSH/LH. The identity of ∼75 kDa band is under investigation. Supported by Grant Agency of the Czech Republic (grant: 15-22765S).

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P033

Determining the effect of extra-villous trophoblast cells on spiral artery remodelling: What is the role of MMP10?

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Background

Spiral artery remodelling is crucial for a successful pregnancy. In a healthy human pregnancy, cells of the placenta called extravillous trophoblasts (EVT) invade into the decidua and interact with the endothelial cells (EC) and vascular smooth muscle cells (VSMC) of the maternal spiral arteries (SA) in a process known as spiral artery remodelling. This process results in the development of low-resistance, dilated SAs that increase the blood supply to the developing fetus. An *in vitro* model has shown several molecules to be upregulated in response to trophoblast conditioned media (TCM) stimulation, including MMP10, an enzyme involved in ECM breakdown, but its role in spiral artery remodelling has yet to be determined.

Aim

The aim of this project was to investigate the role of MMP10 in SA remodelling.

Methods

Experiments were carried out using the human endothelial line SGHEC-7, cultured in 2 ml 5% FCS phenyl-red media. The cells were then stimulated in 0% FCS phenyl red-free media with 100 ng/ml TCM or varying concentrations of IL1β or PMA and incubated for 48 h. ECs were also stimulated with TCM in the presence of an IL1β neutralising antibody. The supernatant was then collected and an R&D Systems human Total MMP-10 (catalogue number: DY910) DuoSet ELISA Development kit was used to detect MMP10. The cell monolayer for each experiment was then frozen and used for subsequent protein determination via Bradford Assay.

Results

TCM was shown to stimulate MMP10 secretion by ECs. MMP10 secreted by ECs increased significantly in a dose respondent manner when stimulated with 0–5 ng/ml of IL1β. Addition of an IL1β neutralising antibody to ECs stimulated with TCM decreased the amount of MMP-10 produced.

Conclusion

This data suggests that IL1β (within TCM) may be stimulating secretion of MMP10 by ECs.

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P034

Geographical variation in canine testicular environmental chemicals: a possible link with altered reproductive development

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Introduction

Environmental chemicals (ECs) are associated with an increased incidence of testicular cancer and reduced sperm quality. Regional differences are thought to reflect different levels of EC exposure. Since the dog is a sentinel of human exposure, we hypothesised that geographical variation in testicular chemical

profiles may be associated with altered reproductive development and/or function.
Methods
Canine adult testes (routine castrations) from UK (West Midlands (WM: $n=12$), East Midlands (EM: $n=9$) and South East England (SE: $n=14$)) and Scandinavian regions (Finland-Vantaa (FV: $n=10$) and Denmark–Copenhagen (DC: $n=10$)) were analysed for ΣPCB, ΣPBDE congeners and DEHP. WM canine ejaculates were analysed for 7 PCB and 7 PBDE congeners ($n=14$; 3 pools: $n=5$, 5 and 4 respectively). Testicular developmental markers were identified by immunohistochemistry: Vimentin (Sertoli cell), PCNA (Proliferation) and DAZL (Spermatogenesis).
Results
Ejaculate: 6 PCB and 4 PBDE congeners detected (PCB-28, 52, 101, 118, 138, 153; PBDE 28, 47, 99, 100). *Testis*: ΣPCB congeners greatest in WM ($P\leq0.0002$), ΣPBDE congeners greatest in FV ($P\leq0.0014$) and DEHP greatest in SE ($P\leq0.0001$). Significant regional differences observed for developmental markers: i) Vimentin: Sertoli cell (SC) numbers ($P\leq0.0001$) and %SC staining ($P\leq0.0001$): EM greater and FV lower for both, ii) PCNA: Scandinavian regions < UK ($P\leq0.0001$), iii) DAZL: FV < DC and UK regions ($P\leq0.0001$). *Testis ECs*: ΣPBDE negative correlation with increased PCNA, DAZL, Vimentin and SC number ($P\leq0.05$, $r=0.43$, 0.43, 0.50, 0.43 respectively), ΣPCB positive correlation with increased PCNA and DAZL cellular expression ($P\leq0.01$, $r=0.46$, 0.47 respectively).
Conclusion
Chemical profiling of ECs in canine testis show i) regional variations and ii) correlations between EC concentrations and the expression of cellular or functional testicular markers. This may account for reported regional variations in male reproductive development.

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P035

Female reproductive ageing: When during oogenesis do chromosomes begin to fall apart?

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Introduction
During female fetal life, mammalian oocytes begin meiosis and arrest in prophase of meiosis I when they become enclosed in primordial follicles. Bivalent chromosomes established during this time have to remain intact until they are resolved during anaphase I, just before ovulation. Bivalents are stabilized throughout this period by Rec8-containing cohesin complexes. In women, however, extending this period for more than 35 years increases the risk of aneuploidy resulting in infertility, miscarriage and birth defects. Previous studies in our laboratory on fully-grown mouse oocytes indicate that the age-related increase of segregation errors during meiosis I is accompanied by the depletion of chromosomal Rec8 levels. This depletion is accompanied by destabilization of the bivalent chromosome architecture. Nevertheless, the mechanisms and timing of cohesin depletion remain unknown.
Methods
We investigate the possibility that premature cohesin loss could be caused by leaky inhibition of the protease separase, which cleaves Rec8 during anaphase, using a separase knockout mouse model. Also, using Rec8-myc mouse model we explore which stage of oocyte development is susceptible to cohesin loss during age.
Results and discussion
Our results indicate that the loss of cohesin during age also occurs in separase deficient mouse oocytes. Additionally, we found that chromosome-associated cohesin is lost from oocytes at the primordial follicle stage. Together, these results suggest that age-related loss of cohesin occurs during the prolonged prophase-arrest at the primordial follicle stage by a separase-independent mechanism.

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P036

Cellular modeling of citrin deficiency using human induced pluripotent stem cell-derived hepatocytes

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Citrin deficiency (CD) is a recessive genetic disorder caused by mutations in *SLC25A13* gene which encodes citrin protein. CD patients manifest various symptoms related to nutrient metabolism such as urea cycle failure, abnormal amino acid levels, and fatty liver. To understand the pathophysiology of CD, the molecular phenotypes were investigated using induced pluripotent stem cells (iPSCs) derived from dermal fibroblasts of CD patient (CD-iPSCs). Here we demonstrate that aberrant mitochondrial β-oxidation may lead to fatty liver in CD patients. Similar to WT iPSCs, CD-iPSCs normally differentiated into hepatocyte-like cells and represented hepatic characteristics, including expression of hepatocyte-specific genes, albumin secretion, glycogen storage and low density lipoprotein (LDL) uptake ability. However, hepatocyte-like cells (HLCs) derived from CD-iPSCs (CD-HLCs) failed to produce urea from ammonia. Cellular triglyceride and lipid granule levels were significantly increased in CD-HLCs compared with WT-HLCs. This symptom was intensified in the presence of high-glucose. PPAR-α and its target genes which are involved in mitochondrial β-oxidation were downregulated in CD-HLCs, and treatment with a PPAR-α agonist partially reduced the lipid accumulation in CD-HLCs. In addition, the mitochondria in CD-HLCs exhibited abnormal morphologies and decreased mitochondrial protein levels. Based on these observations, we suggest that CD-HLCs partly mimic CD patient symptoms *in vitro*, and the lipid accumulation in CD-HLCs accounts for dysfunctional mitochondrial β-oxidation and abnormal mitochondrial structures.

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P037

Disease takes its Toll on reproduction: Toll-like receptors and the bovine corpus luteum

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Introduction
Toll-like receptors (TLRs) are critical mediators of the host defence against pathogens, but may also directly influence reproduction. TLR signalling pathways have been identified in bovine granulosa cells. However, despite key diseases, such as mastitis, being associated with reduced luteal function, TLRs have not been extensively examined in the corpus luteum (CL). TLRs have also been implicated in tissue repair and remodelling processes critical to the rapid tissue changes associated with luteinisation and luteolysis. We hypothesise that the TLRs are expressed in the bovine CL and directly influence luteal function.
Methods
Bovine CL (very early haemorrhagic, early, mid, late/regressing $n=3$–4/stage) were collected at abattoir and morphologically staged. Total RNA was extracted, reverse transcribed and subjected to RT-PCR for TLR1-10. Subsequently, quantitative Taqman PCR was performed for the key receptors TLR2 and TLR4, using housekeeper genes (GAPDH and RPLPO) to normalise transcription levels. All reactions were prepared in triplicate. The housekeepers were tested for stability and a reference CT determined, which was used to calculate delta CT and fold-change values.
Results and discussion
This study demonstrated for the first time that the bovine ovary expresses mRNA encoding TLR1 to 10. Quantitative RT-PCR confirmed that TLR2 and TLR4 were expressed throughout the luteal phase, however no significant difference in the expression of TLR2 or TLR4 mRNA was detected between the different luteal stages (one-way ANOVA $P>0.05$). In addition, TLR4 mRNA was expressed more highly than TLR2 ($P<0.05$). The expression of TLR1-10 mRNA by the bovine CL demonstrated the potential for regulation of luteal function by members of the TLR family. Future studies will investigate how TLR signalling might impact luteal function and how this might be altered in animals with concurrent disease. (Supported by the SRF Vacation Scholarship.)

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P038

The impact of the selective progesterone receptor modulator (SPRM), ulipristal acetate (UPA) administration upon cell proliferation markers within the human endometrium

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Introduction
Selective progesterone receptor modulators (SPRMs) have been reported to decrease cell proliferation within uterine fibroids, and reduce menstrual blood loss. The SPRM Ulipristal Acetate (UPA) has an anti-proliferative effect on fibroid tissue, yet its impact on endometrial cell proliferation is not well understood. The aim of this study was to quantify the effects of the SPRM UPA administration on endometrial cell proliferation within the human endometrium.
Methods
Endometrial biopsies were collected with ethical approval and informed consent from women with uterine fibroids treated with UPA (12/SS/0238). Control proliferative and secretory phase endometrium was sourced from tissue archives (10/S1402/59). Endometrial samples were categorized into three groups ($n=6$ per group): proliferative phase (PP), secretory phase (SP) and UPA-treated endometrium (UPA). Endometrial samples were immuno-stained with two cell proliferation markers, Ki67 and PH-H3. For each proliferation marker, the immuno-stained stromal and glandular cell proliferation indices (CPI), within each tissue group, were measured using an established stereology method. The CPIs from each tissue group were compared, for each cell type, with multiple *t* tests. Statistical significance was adjusted to $P<0.0167$.
Results and discussion
The results show a reduction in Ki67 stained stromal and glandular CPI in the UPA-treated samples compared to the PP samples ($P=0.0238$ and $P=0.0022$ respectively). The results also demonstrate a significant reduction in Ki67 stained stromal and glandular cell CPI in the SP samples when compared to the PP samples ($P=0.0043$ and $P=0.0022$ respectively). These observations are consistent with an anti-proliferative effect of UPA on the human endometrium, which may play a role in the reduction of menstrual blood loss experienced by women prescribed with the SPRM, UPA. (Funding: Medical Research Council (MRC) Centre grant (G1002033) and Tenovus Scotland.)

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P039

Mouse follicles have a smaller follicular antrum in the absence of oocyte core 1-derived O-glycans at two weeks of age

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Introduction
A role for oocyte core 1-derived O-glycans in the regulation of ovarian follicle development has previously been shown using the C1galt1$^{F/F}$:ZP3Cretransgenic mouse model. In this model, oocyte-specific ablation of core 1-derived O-glycans results in a sustained increase in ovulation rate and fertility, which is hypothesized to be due to changes in follicle development. In this study, we sought to characterise the role of oocyte core 1-derived O-glycans in ovarian follicle development by comparing follicle morphology and development between Control (C1galt1$^{F/F}$) and Mutant (C1galt1$^{F/F}$:ZP3Cre) females at 2 weeks of age.
Methods
This study was approved by the Local Ethical Review Panel (University of Oxford). Ovaries were collected from 2 week-old Control and Mutant mice, fixed in formalin, embedded in paraffin, serially sectioned at 5 μm, and stained with haematoxylin. Photographs were taken at ×40 magnification. A set of morphological criteria was defined and used to classify follicles by stage of development, and the proportion of follicles at each stage per ovary calculated. Measurements were taken, using ImageJ software, of several morphological variables including follicle area, antral area and number of granulosa cells per follicle.
Results and discussion
We found that the mean antral area was significantly smaller in 2 week-old Mutant follicles compared with Controls, despite no difference in mean follicle area. Mutant follicles also had an increased number of granulosa cells (whose accumulation is an indicator of follicle development) per follicle at the preantral stage. These results suggest that development of follicles containing oocytes that lack core 1-derived O-glycans is prolonged, delaying antrum formation. This study was partially funded by Nuffield Department of Obstetrics and Gynaecology.

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P040

SIRT1 regulates low oxygen induced bovine granulosa cell proliferation through interaction with VEGF-AKT-mTOR pathway

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Introduction
Proliferation of granulosa cells (GCs) is essential for oocyte growth toward ovulation. Extreme proliferation of GCs is taken place under hypoxic condition due to lack of vascularization in the follicles. However, the mechanisms underlying proliferation of GCs under hypoxia have not been elucidated. In the present study, we examined the effect of low oxygen level on molecular background of cellular proliferation in bovine GCs.
Methods
Bovine GCs obtained from 2 to 5 mm follicles were incubated under 5% (low) and 21% (high) oxygen level for 24 h. To understand comprehensive gene expression, RNA-seq was conducted using next generation sequence technique. From the results of RNA-seq analysis, we compared the expression level of HIF-1α and VEGF using western blotting. Proliferative activity was examined by BrdU intake, and amount of phosphorylated mTOR, AKT, and S6RP were compared under low and high oxygen level. To examine the role of VEGF in the hypoxia-induced cellular proliferation, proliferation activity and phosphorylation of AKT and mTOR was examined using anti-VEGF neutralizing antibody. We examined the effect of oxygen level on expression level of SIRT1 and effects of SIRT1 activation by resveratrol (Res) under low oxygen on proliferative activity and the amount of phosphorylated proliferation related proteins.
Results and discussion
Gene expression analysis revealed upregulation of genes associated with HIF-1α. Low oxygen level increased proliferation activity of GCs, expression level of VEGF, amount of phosphorylated mTOR, AKT, and S6RP with high HIF-1α expression, whereas, anti-VEGF antibody treatment reversed activation of AKT-mTOR pathway and reduced cellular proliferative activity. On the other hand, low oxygen level reduced SIRT1, and activation of SIRT1 by Res decreased cellular proliferation with reduction of phosphorylated mTOR. These results suggest that low oxygen level stimulates the VEGF-AKT-mTOR pathway, which contributes to GC proliferation, and SIRT1 is major regulator of the hypoxia associated cellular proliferation.

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P041

Abstract unavailable.

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P042

Comparative investigation of lipid peroxidation and antioxidant enzymes in relation to semen quality

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Introduction
Lipid peroxidation is considered as an important mechanism of male infertility causing impairment in functioning of spermatozoa. The main aim of the study was to investigate the lipid peroxidation and antioxidant enzymes in relation to semen quality.
Materials and methods
For this study total oxidant status (TOS), total antioxidant status (TAS), paraoxonase (PON), arylesterase and malondialdehyde (MDA) were investigated in seminal plasma and serum of all study subjects by photometric method. Semen quality parameters were also examined following standard protocols.

Results and discussion
We found significantly ($P<0.01$) reduced semen quality parameters in infertile group. The results also showed that TOS and MDA were increased significantly ($P<0.01$) while TAS, PON and arylesterase were found significantly ($P<0.01$) lower in infertile group when compared to fertile group. We also found an increased lipid peroxidation in older ages as compared to younger ages. Moreover, significant ($P<0.01$) correlation was also observed between the studied parameters. We concluded that lipid peroxidation plays a significant role in inducing male factor infertility by disrupting the sperm functions and semen quality.

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P043

Pup sex and body mass of rats raised in different lactation litter sizes affect ghrelin and peptide-YY concentrations

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Introduction
Previous studies have established that litter size during lactation influences body size and adiposity in male rat pups, but female pups and appetite-regulatory hormones have not been studied in this way. Gastrointestinal hormones ghrelin and peptide-YY (PYY) have roles in appetite regulation: high ghrelin levels signal hunger high PYY levels signal satiety. Both hormones are also associated with altered body mass and body composition. Previous findings (SRF 2014) demonstrated that lactation litter size affected levels of appetite hormones in gastrointestinal tissue, but not in plasma. Observed changes in hormone concentrations may have been further influenced by significant differences in pup body size between small and large litters, thus additional data analysis accounting for body masses presented here aims to establish the effects on male and female pups being suckled and raised in different sized litters.
Methods
Male and female littermates from small ($n=4$), control ($n=8$) and large ($n=12$) litter sizes were studied at weaning, with litter sizes adjusted <1 day postpartum pups remained with the dams throughout. Appetite hormone levels were quantified using radioimmunoassay. Statistical analyses were performed on measured appetite hormone concentrations, and on concentrations that were corrected for body mass.
Results and discussion
Pups raised in smaller litters were significantly larger ($P<0.001$). No differences were found in measured concentrations of either ghrelin or PYY in plasma between the litter sizes. Correcting for body mass revealed that large pups raised in small litters had the least circulating ghrelin ($P=0.002$) and PYY ($P<0.001$). Analysing by pup sex further revealed that although plasma ghrelin concentrations were only significantly lower in these larger males ($P=0.023$), PYY levels were significantly lower in both the males ($P=0.011$) and females ($P=0.032$). This work highlights the importance of taking into consideration factors such as body mass and sex when investigating hormones that affect body composition.

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P044

Effect of aflatoxin B1, on sperm vitality, mitochondrial function and acrosome reaction: the bovine model

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Introduction
Aflatoxins are poisonous by-products from soil-borne fungus Aspergillus flavus, which involved in the decomposition of plant materials. Various food products, such as maize, sorghum, millet, rice and wheat, are contaminated with aflatoxins. Aflatoxin B1 (AFB1) is the most toxic aflatoxin and classified as carcinogen and mutagen. The lethality and toxicity extent of AFB1 varies between animals and organs. In the current study we examined the effects on sperm function.
Methods
Sperm isolated from fresh ejaculates or epididymis compartments (head, body or tail) were capacitated *in vitro* for 4 h with 0, 0.1, 1, 10 and 100 μM AFB1. The integrity and functionality of sperm were examined simultaneously by florescent staining at 0, 2 and 4 h of incubation. Following capacitation, acrosome reaction (AR) was induced by Ca^{2+} ionophore and examined by FITC-PSA.
Results and discussion
Sperm plasma viability, expressed by membranes integrity, was reduced in sperm isolated from ejaculate, epididymis (head or tail) and treated with AFB1 (1, 10 or 100 μM $P<0.006$). Similar reduction was observed when sperm isolated from the epididymis body exposed to 10 μM ($P<0.05$). In sperm isolated from the epididymis tail, AFB1 reduced the proportion of sperm with Ca^{2+}-activated AR (100 μM 4 h $P<0.05$), but had no effect on pseudo-AR. On the other hand, AFB1 did not affect induced- or pseudo-AR in sperm isolated from the ejaculate. AFB1 impaired mitochondrial membrane potential ($\Delta\Psi$m) in sperm isolated from ejaculate (10 μM 4 h $P<0.02$) or from epididymis tail (1 μM 2 h $P<0.05$).
The findings expose the harmful effects of AFB1 on sperm viability, acrosome integrity and $\Delta\Psi$m. We postulate that AFB1-induced impairment in these characteristics might further affect sperm fertility competence.

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P045

A Bayesian view of rodent seminal cytokine networks

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Introduction
It is understood that active agents in seminal fluid are key to initiating and coordinating mating-induced immunomodulation. This study aimed to characterise the structure of a network of cytokines whose interactions are thought to underpin this process in rats and mice.
Methods
Seminal fluid, collected from isolated seminal glands, and serum, collected by cardiac puncture, were obtained from sexually mature Wistar rats ($n=20$) and CD1 mice ($n=18$). Samples were profiled for interleukin (IL)-1 alpha, IL-1 beta, IL-2, IL-4, IL-5, IL-6, IL-9, IL-10, IL-12 (p40), IL-12 (p70), IL-13, IL-17, eotaxin, granulocyte-colony stimulating factor (G-CSF), granulocyte macrophage-colony stimulating factor (GM-CSF), interferon (IFN)-gamma, IFN-gamma inducible protein (IP)-10, keratinocyte-derived chemokine (KC), leptin, monocyte chemoattractant protein (MCP)-1, macrophage inflammatory protein (MIP)-1 alpha, MIP-1 beta, regulated on activation, normal T cell expressed and secreted (RANTES), tumour necrosis factor (TNF)-alpha and vascular endothelial growth factor (VEGF) by 24-plex fluid-phase cytometric immunoassay. Bayesian modelling methods were applied to these data to illustrate their relative interrelationships.
Results and discussion
In rats, IL-2, IL-9, IL-12 (p70), IL-13, IL-18, eotaxin, IFN-gamma, IP-10, KC, leptin, MCP-1, MIP-1 alpha and TNF-alpha were significantly higher in serum, whilst IL-1 beta, IL-5, IL-6, IL-10, IL-17, G-CSF and GM-CSF were significantly higher in seminal fluid. In mice, IL-1 alpha, IL-1 beta, IL-2, IL-5, IL-9, IL-12 (p40), IL-12 (p70), IL-13, IL-17, GM-CSF, IFN-gamma, MCP-1 and TNF-alpha levels were significantly higher in serum IL-4, G-CSF, eotaxin, KC and RANTES exhibited the opposite trend. The characterisation of physiological cytokine profiles in seminal fluid using Bayesian models has allowed a more detailed inference of likely inter-mediator causal relationships and their interspecific conservation. These models suggested that MCP-1 plays a key role in coordinating seminal cytokine networks in vivo in both species, in part through its effects on KC and RANTES. (Funding: Ostara Biomedical).

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P046

Dynamic changes in expression and DNA methylation of the astrocyte-specific genes Slc1a2 and Lrg1 during development: implications for preterm birth?

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Introduction
Preterm birth associates with later neurological conditions. Many of the underlying mechanisms are unknown however studies suggest changes in DNA methylation may be important. We have previously identified differential DNA methylation at astrocyte-specific genes, Slc1a2 and Lrg1 in preterm infants. Here we set out to describe the normal developmental patterns of gene expression and DNA methylation of Slc1a2 and Lrg1 in vivo in an animal model and assessed the potential utility of cultured astrocytes to study these changes.
Methods
Astrocytes were isolated from Wistar rat forebrain through neural dissociation and selection of Glast+ cells using Magnetic Activated Cell Sorting, at developmental time points corresponding to human brain development: Embryonic day E20.5 (24 weeks), Postnatal day P1 (28 weeks) and P10 (term). Astrocytes isolated at E20.5 and P1 were cultured for 10 days. Gene expression was analysed using RT-qPCR and DNA methylation using pyrosequencing. Experiments were approved under Home Office project licence (PPL60/7874) after local ethical approval.
Results and discussion
Expression of Slc1a2 increased 58-fold ($P<0.01$) during development and expression of Lrg1 decreased 9-fold from P1 to P10 ($P<0.05$) DNA methylation *in vivo* was low at Slc1a2 and did not change between E20.5/P1 and P10 whereas DNA methylation decreased at Lrg1 over time ($P<0.05$). In culture, Slc1a2 expression was lost but Lrg1 expression was equivalent to P10 in vivo levels. Cell culture altered DNA methylation: levels increased at Lrg1 and decreased at Slc1a2 ($P<0.05$). Thus, our study suggests that cultured astrocytes are not suitable for studying changes *in vitro* alternative options including co-culture systems may provide a suitable alternative. Expression and DNA methylation at Slc1a2 and Lrg1 change during early development during a time period corresponding to early postnatal life in many preterm infants. We hypothesize that these processes may be susceptible to disruption following preterm birth.

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P047

Germ cells, from a mouse model of Premature Ovarian Failure, retain the potential to support follicle development when reaggregated with wildtype somatic cells

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Introduction
Premature ovarian failure (POF) is a condition that affects ~1% of women and is idiopathic in 74–90% of cases. Our mouse model of POF, the Double Mutant (DM), results from oocyte-specific ablation of core 1-derivedO-glycans and complex and hybrid N-glycans. DM females are subfertile at 6-weeks of age and infertile at 9-weeks of age. By 3 months, DM females exhibit POF with ovaries containing fewer developing follicles but more primary 3a follicles. We investigated if 3a follicle development was blocked by assessing if germ cells retained the potential to develop when combined with wildtype somatic cells.
Methods
This study was approved by the Local Ethical Review Panel (University of Oxford). Production of a reaggregated ovary (RO) involves separation and isolation of germ and somatic cells and then combining the two cell types to form a pellet. ROs were generated using germ or somatic cells from Control ($Mgat1^{FF}C1galt1^{FF}$) or DM ($Mgat1^{FF}C1galt1^{FF}$:ZP3Cre) mice at 9-weeks and cells from newborn wildtype mice. ROs were transplanted for 21 days beneath the kidney capsule of an ovariectomised immunocompromised mouse.
Results and discussion
Control-germ-ROs contained follicles at the primary and antral stages of development, and DM-germ-ROs contained follicles at all stages of development indicating the arrest of follicle development was overcome (Controls $n=6$, DM $n=6$). Control-somatic-ROs contained follicles at all stages of development however the number of primary follicles was increased in DM-somatic-ROs (Controls $n=3$, DM $n=3$). Our results suggest that germ cells from DM infertile ovaries retain the potential to develop follicles and this technique provides a potential treatment for POF. Furthermore, our results indicate that DM oocytes are affecting somatic cell physiology, imprinting the 'POF phenotype' and therefore the ability of ovarian somatic cells to sustain follicle development.
This study was funded by a MRC New Investigator grant to SW.

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P048

Effect of temperature on bovine granulosa cells cultured under low oxygen in the presence or absence of melatonin

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Heat stress can impair ovarian function and reduce fertility in cattle. It is correlated with oxidative stress and can induce granulosa cell apoptosis and reduce steroid production. Effects of heat stress can be reversed by melatonin, but studies with ovarian cells have only been evaluated under atmospheric oxygen tension. Here we cultured granulosa cells (GCs) from antral follicles in fibronectin-coated 12-well plates in M199 for up to 144h under physiological (5%) oxygen tension. Treatments commenced after 48h of culture and consisted of two incubation temperatures (37.5 vs 40.0°C) and four melatonin concentrations (0, 20, 200, 2000 pg/ml) in a factorial arrangement. Cell number, steroidogenesis and ROS generation were assessed. Cell number decreased by 144 h of culture ($P=0.028$) at 40.0°C. Melatonin reversed the deleterious effect of high temperature on cell number. However, BAX mRNA expression was greater ($P<0.009$) in GCs cultured at 40.0°C than at 37.5°C by 144 h. Culture temperature did not affect ROS, but melatonin reduced ($P<0.001$) ROS generation at all concentrations tested. Oestradiol (E2 pg/10^5 cells) production increased with time ($P<0.001$) and was not affected by temperature. In contrast, high temperature caused a reduction in progesterone production (P4 ng/10^5 cells $P<0.001$) at 144 h of culture. Similarly, the effect of melatonin treatment depended on temperature melatonin linearly increased P4 production at 37.5°C whilst reducing P4 in cells cultured at 40.0°C. In summary, high temperature reduced cell viability and P4 production by GCs. Under low oxygen melatonin mitigated the negative effect of heat stress on cell number, reduced ROS generation, increased P4 production by GCs cultured at 37.5°C, but reduced P4 production by cells cultured at 40.0°C. The results of this study contribute to our understanding of the effects of heat stress on ovarian function and seasonal variation in cow fertility.
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P049

PGF2alpha regulates the expression of genes involved in embryo-maternal interactions in the porcine endometrium and conceptus cells

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Introduction
Proper interactions between uterus and conceptuses are necessary for establishment of pregnancy and implantation. Key factors in these regulations are prostaglandins (PGs). Recently we presented an important role of prostaglandin F2α (PGF2α) and its receptor (PTGFR) in porcine endometrium during early pregnancy. The aim of present study was to determine whether PGF2α can regulate the endometrial and embryonic expression of biglycan (BGN), metalloproteinase-9 (MMP9), transforming growth factor 3 (TGFB3), interleukin-1α (IL1A) and interleukin-6 (IL6) genes that are potentially involved in embryo-maternal interaction during early pregnancy in the pig.
Methods
Endometrial explants collected from gilts on day 12 of the estrous cycle were incubated with PGF2α (100 nM, 1 μM) or vehicle (M199 with 0.1% ethanol) for 24 h at 37°C in a humidified atmosphere (95% air and 5% CO2). After incubation, explants were snap-frozen in the liquid nitrogen. Similarly, cells of conceptuses collected from gilts ($n=5$) on day 14 of pregnancy were treated with PGF2α (100 nM, 1 μM) or vehicle for 24 h at 37°C in a humidified atmosphere (95% air and 5% CO2). After incubation cells were lysed and snap-frozen. Total RNA was isolated from endometrial explants and conceptus cells. The expression of BGN, MMP9, TGFB3, IL1A and IL6 genes was analyzed by real-time PCR.

Results and discussion
In endometrial explants, 1 μM PGF2α elevated gene expression of BGN, MMP9, TGFB3 and IL1A ($P<0.05$). 100 nM PGF2α elevated endometrial expression of BGN and IL1A ($P<0.05$). In conceptus cells, 1 μM PGF2α increased BGN expression ($P<0.05$), while IL1A, MMP9 and TGFB3 was down-regulated by 1 μM PGF2α ($P<0.05$). There was no effect of PGF2α on IL6 expression in porcine endometrial explants and conceptus cells.
Summarizing, differential regulation of BGN, MMP9, TGFB3 and IL1A gene expression by PGF2α in the endometrium and conceptus may promote endometrial remodeling and embryo-maternal communication during early pregnancy.
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P050

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P051

The effect of macrophage colony-stimulating factor (CSF1) on piglet gonad development

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Introduction
CSF1 is the primary growth factor required for macrophage proliferation and differentiation. In adults, the receptor is expressed only in macrophages and their precursors. In mice, mutation of the CSF1 gene produces both male and female infertility due to diminished gonadal steroid production. We tested the effect of increased availability of CSF1 on gonadal development in pigs using CSF1-Fc, a CSF1 fusion protein with increased circulating half-life.
Methods
Testicular and ovarian tissue was obtained from 11 9-week old male and female Large White piglets: (CSF1-Fc (0.75 mg/kg) $n=6$, PBS $n=5$) for haematoxylin and eosin staining and immunohistochemistry (IHC) using Ki67 as a proliferation marker. Testicular macrophages were detected by IHC using CD163.
Total cell count (TCC), Seminiferous tubules, Seminiferous tubule diameter, Leydig cells (LC), Sertoli cells, Germ cells, proliferating cells (PC) and macrophages were quantified in testis samples ($n=5$). The number of ovarian follicle types (FT) and PCs were quantified in ovarian samples ($n=6$).
Results and discussion
The number LCs as a proportion of TCC increased in CSF1-Fc treated animals (means.e.m. 0.170.002) compared to PBS controls (0.110.016 $P=0.026$). Proportion of PCs and LCs compared to TCC also increased in CSF1-Fc treated animals (0.040.0025) compared to PBS controls (0.020.0007 $P=0.021$). Proportion of proliferating LCs increased in CSF1-Fc treated animals (0.0250.0021) compared to PBS controls (0.0120.0004 $P=0.025$). No differences were detected in other testis parameters.
In ovarian samples, proportion of multi-oocyte follicles compared to total follicle count significantly increased in CSF1-Fc treated animals (0.090.022) compared to PBS controls (0.020.0017 $P=0.042$). No differences in number of PCs or other FTs were detected.
These findings suggest that the availability of CSF1 contributes to gonadal cell proliferation in both sexes. The most striking differences occur in males consistent with previous evidence of the role of macrophages in control of LC function.

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P052

Histological categorization of equine ovarian follicles from healthy and diseased mares

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Antral follicle waves in mares are considered a model for ovarian function in women, and can be sampled with relative ease in vivo or following ovary removal to investigate the regulation of follicle growth. To begin determine the effects of disease which may dysregulate follicular granulosa (GC) or theca cell (TC) proliferation, this study characterised 41 antral follicle wall H&E sections recovered from ovaries of 16 mares that were classified as healthy or suffered from chronic mild or severe clinical disease.
Follicles measured on average 21 mm in diameter which was unaffected ($P>0.05$) by disease status, and were allocated to very healthy (VH), healthy (H), early atretic (EA) and late atretic (LA) categories based on the histological appearance of the GC and TC layers. Disease status had no influence on the follicle category ($P>0.05$), and did not alter percentages of basal, intermediate or antral GC, or GC layer thickness ($P\geq 0.3$). However, the TC layer thickness was increased ($P<0.01$) in follicles from mares with severe disease compared with healthy mares. More healthy than diseased mares were in seasonal transition at ovary recovery ($P<0.05$), and transitional follicles showed reduced ($P<0.05$) TC layer thickness and percentages of basal GC and large TC, compared with follicles recovered in deep anoestrus.
Follicle categorization into VH, H or EA did not alter any GC layer characteristics ($P>0.05$), but H follicles had a thicker TC layer than EA follicles ($P<0.05$). In LA follicles the GC layer had disappeared, and the percentage of large TC was reduced, while the percentage of small TC was increased compared to VH and H follicles ($P<0.001$), reducing the TC layer thickness in LA versus H follicles ($P<0.05$).
In conclusion, basic histomorphological GC and TC measurements in equine large antral follicles appear more influenced by season and follicle atresia than by disease.

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P053

The effect of dietary protein level on bovine follicular dynamics in beef heifers

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Introduction
Periconception dietary protein can affect fertility in cattle. This study investigated the effect of dietary crude protein intervention on gene expression in granulosa cells, metabolite concentrations in follicular fluid and serum anti-Müllerian hormone (AMH) concentrations.
Methods
Non-pregnant Angus cross heifers ($n=320$) were group fed an isocalorific low (LP 10%) or high (HP 14%) crude protein diet for >60 days prior to slaughter. Serum was prepared from whole blood collected at exsanguination. Ovaries were collected and antral follicle count (AFC) recorded. Follicular fluid and granulosa cells were collected from healthy medium-sized follicles (4–9 mm). Serum AMH concentrations were measured by ELISA. Metabolite concentrations were measured using a Randox RX-IMOLA autoanalyser. RNA was extracted from granulosa cells (GC). Next-generation sequencing of GC was completed using the Illumina platform and differential genes identified using theTuxedo bioinformatics pipeline.
Results and discussion
AFC was positively correlated with circulating AMH concentrations, with increased AMH concentrations in the LP diet ($P<0.05$). Albumin concentrations were elevated in the follicular fluid of the HP treatment ($P<0.05$), however urea concentrations were lower ($P<0.01$). Gene expression analysis of GC identified 232 differentially expressed genes (>twofold change, $P<0.05$) with a more stringent analysis revealing 12 genes down-regulated and 26 genes up-regulated in the HP treatment ($P<0.05$). Gene ontology (GO) analysis showed that genes were enriched in GO terms including response to external stimulus, proteinaceous extracellular matrix and extracellular matrix structural. These genes are associated with the AP-1 transcription factor network (regulation of cell proliferation and differentiation) and focal adhesion pathways (cell migration and signal carriers). These pathways included genes such as STAR and IGFBP5.
In conclusion, periconceptional dietary protein was observed to affect AFC and

was associated with altered GC gene expression and follicular fluid metabolites.
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P054

Inhibition of macrophage colony-stimulating factor-1 (CSF-1) receptor signalling: a novel therapeutic target for tubal ectopic pregnancy

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Introduction
An ectopic pregnancy (EP) is defined as a conceptus implanting outside of the uterine cavity, most commonly in the Fallopian tube. It is a life-threatening gynaecological condition with limited treatment options. Large and unstable EP are managed by surgical excision. Smaller stable EP are medically managed with the chemotherapeutic drug methotrexate. Methotrexate has considerable side effects and a high treatment failure rate (∼30%). There is an unmet medical need for better-tolerated and more efficacious medical treatments for EP. *In-vitro* studies, data from knockout mice, and human ex-vivo studies suggest that colony-stimulating factor-1 (CSF-1) is essential for the survival of an early pregnancy. We hypothesise targeting CSF-1 receptor signalling may provide a novel therapeutic target for the medical treatment of EP.
Methods
CSF-1R expression was examined in tubal implantation site biopsies obtained from women undergoing surgery for EP ($n=4$) and in an immortalised human first trimester trophoblast cell line (SW.71) by immunohistochemistry and immunocytochemistry. SW.71 cells were exposed to CSF-1 and a CSF-1R antagonist (GW2580) (with and without CSF-1) at different time points (24 and 48 hours) and at a range of concentrations. Proliferation was measured using an MTT assay.
Results and discussion
CSF-1R was expressed abundantly in the syncytiotrophoblast and cytotrophoblast at tubal implantation sites from women with EP. CSF-1R was also expressed in the SW.71 cells. Exogenous CSF-1 (100 ng/ml) increased proliferation of SW.71 cells ($P<0.0001$). GW2580 decreased proliferation of SW.71 cells at concentrations 10, 20 and 40 μM after 48 h ($P<0.01$, $P<0.0001$, $P<0.0001$ respectively). GW2580 also decreased proliferation in SW.71 cells with prior exposure to CSF-1 at 20 and 40 μM after 48 h (both $P<0.0001$).
Conclusion
CSF-1R is expressed in trophoblast cell populations at tubal EP implantation sites. Antagonism of CSF-1 decreases trophoblast cell proliferation. This supports the potential to target CSF-1R signalling as a therapy for EP.

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P055

Putative role for progesterone in *Monodelphis domestica* embryogenesis and pregnancy

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Introduction
Progesterone (P) has an unusual role in didelphid marsupials in that pheromonally induced levels of pro-estrous P trigger ovulation. Work in our lab reveals that serum P levels are significantly higher on pregnancy day 3 in the didelphid, *Monodelphis domestica*, plummet to extremely low levels during pregnancy day 5, and return to day-3 levels on day 9, before gradually declining as pregnancy advances. Because this precipitous decline coincides with the highest levels of embryonic mortality during the 14-day gestation period in *M. domestica*, we investigated the possibility that these unusual events may be related.
Methods
We compared uterine histological samples from days 1, 3, 5, 7, 9, 11 and 13 of pregnancy as well as samples from non-pregnant females. To understand the pattern of progesterone receptor (PR) expression in the uterus during pregnancy, we used HRP-mediated immunoassay of histological samples from these pregnancy stages.
Results and discussion
Our results indicate that P levels fluctuated in a manner coincident with that of thickness of the endometrial epithelium, both parameters being highest on days 5 and 9. Additionally, on day 9, the uterine stroma as well as the uterine glands in them were at their thickest. PR was clearly detectable in the cytoplasm and stroma of uterine gland cells at all pregnancy stages, declining gradually as pregnancy progressed, with one Xion: day 5. PR expression was extremely faint, if at all detectable, at this pregnancy stage. Low PR expression would thus seem to exacerbate the effects of low P on day 5 of pregnancy, suggesting that low or absent progesterone signaling may have adverse effects on embryonic survival.

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P056

Male infertility-linked point mutation dramatically reduces the Ca^{2+} oscillation-inducing activity of sperm PLCzeta without affecting its ability to hydrolyse PIP2

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Introduction
Sperm-specific phospholipase C zeta (PLCζ) is widely considered to be the physiological stimulus that evokes intracellular calcium (Ca^{2+}) oscillations that are essential for the initiation of egg activation and early embryonic development during mammalian fertilization. Sperm-delivered PLCζ hydrolyses phosphatidylinositol 4,5-bisphosphate (PIP_2) stores within the ooplasm, triggering Ca^{2+} oscillations through the inositol 1,4,5-trisphosphate (IP_3) signaling pathway. PLCζ consists of four EF hand domains at the N-terminus, the characteristic X and Y catalytic domains in the centre, followed by a C-terminal C2 domain. A recent genetic study reported a male infertility case that was directly associated with a point mutation in the PLCζ C2 domain, where an isoleucine (I) residue (I489) had been substituted with a phenylalanine (F). Herein, we have analysed the effect of this mutation on the *in vivo* Ca^{2+} oscillation-inducing activity and the *in vitro* biochemical properties of human PLCζ.
Methods
For comparative analysis, bacterially-expressedrecombinant proteins or cRNA encoding luciferase-tagged versions of wild-type and $PLCζ^{I489F}$ mutant were microinjected into unfertilised mouse eggs. The enzymatic and biochemical properties of $PLCζ^{WT}$ and $PLCζ^{I489F}$ mutant were analysed using an *in vitro* [^{3}H] PIP_2hydrolysis and liposome binding assays.
Results and discussion
Microinjection of cRNA or recombinant protein corresponding to $PLCζ^{I489F}$ mutant at physiological concentrations completely failed to cause Ca^{2+}oscillations in eggs. However, this infertile phenotype could be effectively rescued by microinjection of relatively high (non-physiological) amounts of recombinant mutant $PLCζ^{I489F}$protein, leading to Ca^{2+} oscillations and egg activation. Our *in vitro* biochemical analysis suggested that the $PLCζ^{I489F}$ mutant displayed similar enzymatic properties, but dramatically reduced binding to PI(3)P and PI(5)P-containing liposomes, compared to wild-type PLCζ. Our findings highlight the importance of PLCζ at fertilization and the vital role of the C2 domain in PLCζ function due to its direct interaction(s) with either PI(3)P, PI(5)P or other unidentified egg proteins.

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P057

Antigen unmasking improves visualisation efficacy of phospholipase C zeta (PLCζ) in mammalian sperm to enable diagnostic applicability for evaluating PLCζ-dependent human oocyte activation deficiency

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Introduction
Mammalian oocyte activation is mediated via a series of intracellular calcium oscillations induced by a sperm-specific phospholipase C, PLCzeta (PLCζ). PLCζ

presents significant promise as a clinical therapeutic for some forms of male infertility. However, the utility of PLCζ as a potent diagnostic tool for human sperm remains undefined. Furthermore, considerable variation in reported PLCζ localization patterns in sperm highlight the necessity for improvement in antibody specificity and detection protocols.

Materials and methods

Two PLCζ antibodies were employed in mouse, porcine, and human sperm. Human sperm was obtained following informed, written consent with full ethical approval. Antibodies against sperm-specific proteins, PAWP and acrosin, were used as controls. Ejaculated human sperm ($n=15$) was subject to density gradient washing. Mouse sperm ($n=3$) was obtained via epididymal puncture, while porcine sperm ($n=3$) was supplied commercially. Aldehyde- or methanol-fixed sperm were subject to PLCζ immunofluorescent analysis (>300 cells, $n=3$) following either HCl exposure (pH=0.1–0.5), acid Tyrodes solution (AT) exposure (pH=2.5), or heating in 10mM sodium citrate solution (pH=6.0).

Results and discussion

Despite high specificity of antibodies to native PLCζ following immunoblotting, immunofluorescent visualization efficacy in sperm from all three species was poor, whereas post-acrosomal WW-binding protein (PAWP) and acrosin exhibited relatively impressive results. Antigen unmasking/retrieval protocols (AUM) on aldehyde-fixed sperm significantly enhanced visualization efficacy, but exerted no significant change upon PAWP or acrosin fluorescence. Furthermore, AUM enhanced PLCζ visualization efficacy in methanol-fixed sperm. This suggests that poor PLCζ visualization efficacy may be due to strong interactions of PLCζ, occluding antibody access. Finally, examination of sperm from individual donors revealed that AUM differentially affects observable PLCζ parameters in sperm from different males, suggesting a risk of potential misdiagnosis without application of AUM.

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P058

The effect of transforming growth factor B on luteal angiogenesis and function *in vitro*

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Introduction

The formation of a functional corpus luteum (CL) is integral to the establishment and maintenance of pregnancy. The development of the CL requires tightly regulated angiogenesis, which is controlled by a plethora of pro and anti-angiogenic factors. Transforming growth factor B (TGFB) has been identified as a potential mediator of this process. This study tested the hypothesis that TGFB would adversely impact on endothelial cell (EC) development and reduce the steroidogenic capacity of bovine luteal cells *in vitro*.

Methods

Bovine luteal cells from early CL ($n=4$) were cultured in a physiologically relevant system and treated with TGFB (0, 1, 10 ng/ml). Treatment commenced on day 1 or 5, cells were fixed 4 days later. Von Willebrand factor, VE-cadherin and smooth muscle actin immunohistochemistry were utilised to identify areas of EC and mural cell growth. Total area and perimeter of EC networks as well as the number of individual EC clusters were quantified by image analysis. Spent culture media was collected for measurement of progesterone and fibroblast growth factor 2 (FGF2) concentrations by ELISA.

Results and discussion

TGFB reduced ($P<0.001$) the area, perimeter and number of EC networks formed at both time points. Indeed, these were completely abolished by TGFB at 10 ng/ml. Similar effects of TGFB were observed on VE-cadherin EC networks, with a clearly visible reduction in the number and size of networks throughout culture at both TGFB doses. TGFB appeared to increase mural cell growth in a dose dependent manner at both time points, however this was not quantified. Spent media progesterone concentrations were decreased tenfold by TGFB on both day 5 and 9 ($P<0.001$). However, TGFB had no impact on FGF2 concentrations on days 5 and 9. In conclusion, TGFB inhibited luteal angiogenesis and progesterone production whilst promoting mural cell development *in vitro*.

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P059

Insight into the molecular mechanisms underlying enhanced gonadotropin hormone receptor activity in polycystic ovarian syndrome

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Introduction

Polycystic ovary syndrome (PCOS) is a common endocrine disorder, affecting 5–10% of women of reproductive age, and is the major cause of anovulatory infertility and hyperandrogenism. Aberrant secretion and or action of gonadotropins are implicated but, to date, we have only limited knowledge about the precise mechanisms that are involved. Recent genome wide association studies have discovered significant signals have emerged at loci close to the genes on chromosome 2 coding for the gonadotropin receptors. The functional significance of these polymorphisms is, as yet, far from clear and this represents a key area for further research.

Methods

In this study granulosa-lutein (GL) cells were obtained from women with and without PCOS undergoing IVF. RNA was extracted and quantitative real-time PCR was performed to analyse differential gene expression. Cyclic AMP production was measured after administration of luteinising hormone (LH) and follicle stimulating hormone (FSH) to cultured cells using a second messenger accumulation assay. Intracellular calcium signalling was measured in cultured cells after administering LH using calcium fluorescent indicators.

Results

Increased expression of full-length FSH ($P=0.02$) but not LH receptor RNA was seen in PCOS, along with increased expression of signaling and trafficking molecules including β arrestin 2 ($P=0.03$), PDZ protein GIPC ($P=0.07$) and adaptor protein containing PH domain, PTB domain and leucine zipper 1 (APPL1) ($P=0.005$). No significant differences were seen in expression of LH receptor splice variants. Cyclic AMP level measured after administration of LH for 5 min was higher in cells from women with PCOS than from control women (×fourfold increase). Cyclic AMP measured after administration of FSH for 5 min however was negligible in both groups, suggesting involvement of an alternative to the traditional Gs signaling pathway. Administration of LH activated a calcium signaling response in granulosa cells.

Conclusion

These provisional results reveal multiple molecular alterations of LH receptor action and downstream signaling in PCOS.

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P060

Effect of *in vitro* fertilization (IVF) and embryo culture duration on mouse development and postnatal health

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Since the advent of IVF (*in vitro* fertilisation), several million babies have been born worldwide. However, reports link *in vitro* techniques with adverse short and long-term health outcomes. Using a mouse model, we investigated the effect of IVF and duration of culture on blastocyst development and cell number and the postnatal health of offspring. Experimental groups (8–13 litters each): NM (natural mating, non-superovulated) IV-ET-2Cell (2-cell embryos derived *in vivo* from superovulated mothers (SOM) and immediately transferred (ET) to recipients IV-ET-BL (blastocysts derived *in vivo* from SOM and immediate ET) IVF-ET-2cell (2-cell embryos generated by IVF from SOM, short culture and ET) IVF-ET-BL (blastocysts generated by IVF from SOM, long culture and ET). IVF blastocysts after prolonged culture developed slower and comprised reduced trophectoderm and ICM cell numbers compared with *in vivo* generated blastocysts ($P<0.05$ $n=50$–87 per treatment). IV-ET-2Cell ($n=57$), IV-ET-BL ($n=47$), IVF-ET-2Cell ($n=75$) and IVF-ET-BL ($n=42$) groups compared with NM controls ($n=80$), showed increased body weight, increased SBP, impaired GTT and abnormal organ:body weight ratios in both genders ($P<0.05$), independent of litter size. At weeks 15, 21, SBP for IVF-ET-BL males was increased compared to IV-ET-BL and IVF-ET-2Cell males. However, glucose concentration 2 h after glucose injection and AUC (area under curve) in male IVF-ET-BL was reduced compared with IVF-ET-2Cell males. Serum insulin for IVF-ET-BL males was significantly reduced compared with IVF-ET-2Cell, but serum glucose and G:I ratio did not show any significant differences. No differences were evident between the four treatments groups for females. We

conclude that reproductive treatments affect the development and potential of preimplantation embryos, influencing postnatal development and physiology compared with undisturbed reproduction. In particular, duration of embryo culture, with normalised SO, IVF and ET, may affect male offspring cardiometabolic health and organ allometry but female health is less sensitive.

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P061

Altered expression of genes affecting oestrogen metabolism and action in granulosa-lutein cells of women with PCOS

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Introduction
Polycystic ovary syndrome (PCOS) is a common endocrinopathy in premenopausal women and is associated with hyperandrogenism, anovulatory infertility, and metabolic abnormalities. Abnormalities in the steroidogenic pathway of both theca and granulosa cells have previously been reported, but there has been no comprehensive analysis of steroidogenic gene expression in granulosa-lutein (GL) cells of women with PCOS. In this study, we investigated a panel of genes involved in steroid synthesis, metabolism and action in GL cells of women with PCOS compared to women with normal ovaries and regular cycles.
Methods
Granulosa cells were collected during IVF from women with and without PCOS. RNA was extracted, cDNA synthesised and quantitative PCR used to screen 19 genes. Expression data was also correlated to the FSH dose used for superovulation. Further, we examined the effects of FSH or the androgen dihydrotestosterone (DHT) on expression profiles in cultured GL cells to determine whether any differences observed could be attributed to the direct actions of FSH or androgen.
Results/discussion
The majority of steroidogenic genes were unchanged in GL cells of women with anovulatory PCOS, but CYP11A1 expression was significantly decreased (threefold, $P<0.01$). However, the most significant differences were seen in genes involved in oestrogen action. SULT1E1 (encoding oestrogen sulfotransferase) was significantly increased sevenfold ($P<0.001$), with similar results found in ovulatory women with polycystic ovary morphology. In addition, expression of ESR1 and ESR2 (encoding ERα and ERβ) was increased threefold ($P<0.05$). Preliminary *in-vitro* studies showed that SULT1E1 expression is not altered by FSH but is upregulated by DHT treatment.
These results are the first to show that genes involved both in oestrogen metabolism and action are differentially expressed in ovarian cells from women with PCOS. It remains to be determined what the significance of these findings is in the aetiology of reproductive dysfunction in PCOS.

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P062

Abstract withdrawn.

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P063

Preantral follicle development in cultured reaggregated neonatal ovaries

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Introduction
Follicle development is complex, and the use of reaggregated ovaries (ROs) allows us to investigate oocyte-somatic cell interactions, since they can be created using different sources of germ and somatic cells. Production of an RO involves the separation of germ and somatic cells using differential plate adhesion, followed by reaggregation into a pellet. The pellet is then transplanted beneath the kidney capsule of an immunocompromised mouse which facilitates follicle development in the ROs however, development cannot be observed. We have developed an *in vitro* technique that supports RO growth in order to observe follicle development over time.
Methods
This study was approved by the Local Ethical Review Panel (University of Oxford). ROs generated from 4 to 5 neonatal mice (aged P0-P6) were cultured in Waymouth media supplemented with FSH, insulin-transferrin-selenium, ascorbic acid and FBS, for 7 and 14 days. ROs were also transplanted beneath the kidney capsule of an immunocompromised mouse for 21 days. ROs were embedded, sectioned, H&E stained and follicle development assessed.
Results and discussion
ROs cultured for 7 days ($n=3$) contained primary follicles, whereas after 14 days of culture ($n=3$), ROs contained primary, secondary and preantral follicles. ROs developed *in vivo* for 21 days contained primary, secondary, preantral and antral follicles ($n=4$). The presence of antral follicles in the *in vivo* developed RO demonstrates the potential for full follicle development in cultured ROs. Although further studies are needed to enable follicles to develop to the later stages *in vitro*, RO culture provides us with the ability to observe follicle development in real time, which is hugely advantageous to furthering our understanding of follicle function.
This study was partially funded by Nuffield Department Obstetrics and Gynaecology.

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P064

First evidence of a menstruating rodent: the spiny mouse (*Acomys cahirinus*)

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Background
Menstruation, the cyclical breakdown of the superficial endometrial layer in the absence of pregnancy, occurs in 1.5% of mammals. There is no obvious phylogenetic link between species known to menstruate: humans, Old World monkeys, some bats and the elephant shrew, and true menstruation has never before been reported in rodents. Observations of blood at the vaginal opening in some females, led us to examine the possibility that the spiny mouse (*Acomys cahirinus*) menstruates.
Methods
Virgin spiny mice ($n=14$, 12–16 weeks) were samples by daily vaginal lavage for two complete cycles. Stage-specific collection of reproductive tissues and plasma was used for comparative histology, and ELISA assay for plasma progesterone. Decidualised endometrial stromal cells were detected using prolactin immunohistochemistry.
Results
Blood was present in vaginal lavages of all females (14/14) during the transition frm the luteal to the follicular phase in both cycles. Mean cycle length was 8.70.4 days with red blood cells seen in the lavages over 3.00.2 days. The endometrium was thickest during the luteal phase, when plasma progesterone peaked at ∼102 ng/ml and the optical density for prolactin immunoreactivity was strongest. Immunopositive endometrial cells were absent during the follicular phase, and shed at the time of vaginal bleeding. Blood and endometrial shedding were seen in the uterine lumen at the conclusion of each infertile cycle. These menstrual changes occurred in association with regression of the corpora lutea in the ovary.
Discussion
The spiny mouse is the first rodent to show spontaneous decidualisation and menstruation. This discovery contradicts existing opinion that rodents do not menstruate, and challenges the evolutionary theories of menstruation. The spiny mouse provides a novel research species to advance our understanding of human menstrual and endometrial pathophysiology, and perhaps menopause.

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