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SRF 2015

SYMPOSIA

ORAL COMMUNICATIONS

Symposia

Symposia 1: The sperm race

S001

Occupational and environmental influences on semen quality

Allan Pacey[1], Andy Povey[2], Roseanne McNamee[2], Harry Moore[1] & Nicola Cherry[3]
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The effect of occupation and environment on semen quality is of obvious interest to doctors and their patients. The National Institute for Health and Care Excellence (NICE) suggest that men should be advised about the possible negative effects of: i) some occupations; ii) cigarette smoking; iii) alcohol consumption; iv) tight underwear; v) recreational drugs; and vi) high BMI. However, the evidence that underpins these suggestions is often quite weak, relying on small underpowered studies that inadequately control for confounding variables. By contrast, the Chemicals and Pregnancy Study UK (CHAPS-UK) was a comprehensive investigation of over 2 200 men attending 12 fertility clinics across the UK for their first semen analysis. It used a case-referent design to examine occupational and lifestyle risk factors for low motile sperm concentration (<12 million motile sperm per ml) and low sperm morphology (<4% normal forms). The results suggest that occupational risks for both low motile sperm concentration and/or low sperm morphology include exposure to paint strippers, lead and glycol ethers. By contrast modifiable and non-modifiable lifestyle factors related to low motile sperm concentrations include: i) a history of previous testicular surgery; ii) being in manual work or not working; iii) black ethnicity; iv) wearing of boxer shorts; and v) previous paternity. Lifestyle factors relating to low sperm morphology were: i) sample production in the summer; ii) cannabis use; and iii) sexual abstinence. These data challenge the general guidance given in NICE guidelines and suggest that a revision may be required.

DOI: 10.1530/repabs.2.S001

S002

How sperm spot the egg: chemosensation in sea urchin and human sperm

Timo Strünker
Max Planck Institute, Bonn, Germany.

The origin of a new organism requires the fusion of the female's oocyte with the male's sperm. The oocyte increases the chance for an encounter with a sperm by the release of chemoattractants, which provide chemical cues for the sperm to spot the egg. This mechanism termed 'sperm chemotaxis' is important for fertilization in many species. Sea urchins have served as a model organism for fertilization, in particular for sperm chemotaxis, since the early 20th century. Sea urchin sperm provide a powerful model to unveil both the cellular principles underlying chemosensation at the physical sensitivity limit and the computational operations performed by sperm during chemotactic steering. Whereas sea urchin sperm rely on chemotaxis, mammalian sperm seem to navigate along both chemical and physical cues within the female genital tract; chemo-, thermo-, rheotaxis, and combinations thereof have been proposed. Some signalling components are conserved between sea urchin and mammalian sperm, but the sensing mechanisms are distinctively different. In fact, in mammalian sperm, the navigation mechanism(s) and underlying signaling pathways are rather ill-defined, due to the demanding challenge to experimentally emulate the complex chemical, hydrodynamic, and topographical landscape of the female genital tract.

DOI: 10.1530/repabs.2.S002

S003

Gamete interactions in the female reproductive tract: it is not a race!

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Until recently the mammalian oviducts or fallopian tubes were regarded as inert organs that provide a passive racecourse where spermatozoa compete against each other to reach the egg. Recent evidence suggests, however, that the female reproductive tract recognises the arrival of spermatozoa and initiates changes in gene expression and protein secretion profiles. We have observed that X- and Y-chromosome bearing spermatozoa alter gene expression in the maternal tract in different ways. This observation suggests that the female reproductive tract can sense the genetic characteristics of the spermatozoa. We hypothesise the existence of alternative scenarios: either the female reproductive tract is equipped with suitable sensory mechanisms, or the spermatozoa send out specific signals according to their genetic makeup to manipulate the maternal milieu for their own benefit. We need to develop tools with high precision to prove the validity of these hypotheses. However, one point is clear. Sperm transport in the female reproductive tract is not a simple race, where whoever runs faster wins the game and reaches the egg, as recently depicted in the popular media. It is a highly complex series of events. Understanding these interactions may help us learn the fundamental aspects of intercellular communication which induces short-term immune-tolerance.

DOI: 10.1530/repabs.2.S003

SRF-SRB Exchange Lecture

S004

SRF–SRB Exchange Lecture: Musashi RNA binding protein MSI2 interacts with SFPQ and controls the expression of target mRNAs *Tbx1* and *Piwil1* in male germ cells during spermatogenesis

Jessie M Sutherland[1], Barbara A Fraser[1], Alexander P Sobinoff[1], Nicole A Siddall[2], Peter Koopman[3], Gary R Hime[2] & Eileen A McLaughlin[1]
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Controlled gene regulation during gamete development is vital for maintaining reproductive potential. During the process of gamete development, male germ cells experience extended periods of inactive transcription despite requirements for continued growth and differentiation. Spermatogenesis therefore provides an ideal model to study the effects of post-transcriptional control on gene regulation. During spermatogenesis post-transcriptional regulation is orchestrated by abundantly expressed RNA-binding proteins (RBPs). One such group of RBPs is the Musashi family, previously identified as critical regulators of testis germ cell development and meiosis in *Drosophila,* and also shown to be vital to sperm development and reproductive potential in the mouse. Herein, we focus in depth on the role and function of the vertebrate Musashi ortholog: Musashi-2 (MSI2). Through detailed expression studies and utilising our novel transgenic *Msi2* testis-specific over-expression model we have identified two unique RNA-binding targets of MSI2 in spermatogonia, *Piwil1* and *Tbx1*, and have demonstrated a role for MSI2 in the regulation of mature mRNA localisation and expression. We have also provided evidence to suggest that splicing protein, SFPQ, acts in complex with MSI2, exclusively in the nucleus of spermatocytes and spermatids, functioning in pre-mRNA processing. This firmly establishes MSI2 as a master regulator of post-transcriptional control during post-mitotic spermatogenesis and highlights the significance of the sub-cellular expression of RNA binding proteins in relation to their function.

DOI: 10.1530/repabs.2.S004

Symposia 2: Surgical intervention and fertility

S005

Ovarian autografts and autotransplants as experimental and translational models for the treatment of infertility

Bruce K Campbell
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In contrast to the testis, the ovary is an internal organ which contains a finite supply of primordial follicles, enclosed by ovarian somatic cells. Development of ovulatory follicles from this primordial pool capable of releasing a developmentally competent oocyte is an extremely complex developmental process which involves interactions between an array of local factors, the pituitary gonadotrophins and other endocrine factors. Elucidation of these local and endocrine mechanisms and the translational application of this knowledge to enable the treatment of infertility and preservation of fertility has been greatly complicated by the internal location of the ovary within the abdominal cavity. Surgical intervention models, such as ovarian and uterine autotransplants, were initially developed to overcome these issues by facilitating access to the reproductive tract and associated vasculature for mechanistic research. These experimental models proved pivotal in the determination of the basic endocrinology of the ovulatory reproductive cycles, the study of the relative roles of the gonadotrophins in the control of the terminal stages of ovulatory

follicle development and the modulatory role that local factors played in this stimulatory process. Surgical skills developed as part of this process were in turn pivotal in the successful utilisation of cryopreserved ovarian tissue for ovarian autografting and/or whole ovarian autotransplantation as a potential means to restore fertility to women at risk of premature ovarian failure. In this presentation, the development and applications of these experimental models will be reviewed prior to discussion of the efficacy and application of these related strategies for fertility preservation.

DOI: 10.1530/repabs.2.S005

S006

Uterus transplantation: an update

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The last frontier to conquer in female infertility is absolute uterine factor infertility (AUFI), affecting more than 10 000 women in the UK. Uterine transplantation (UTx) is now the first available treatment for this large group of women. Adoption and gestational surrogacy are other means to obtain motherhood, but the acceptances of these arrangements in the society vary greatly between societies.

Our research group initiated a step-by-step developmental animal-based research approach on UTx in 1999 and have optimized all aspects of the procedure in several animal species. Today 11 human UTx ttempts have been made, with the last nine of them performed by our team. The first two human UTx-attempts, which were unsuccessful, were done in Saudi Arabia in 2000 and in Turkey in 2011.

In early 2013 our team completed the surgeries of a series of totally nine human UTx, with live uterus donors. Eight recipients were MRKH patients and one had undergone a hysterectomy because of cervical cancer. The mean age of the recipients was 31.5 ± 3.9 years. Five donors were mothers and others were close relatives and in one case family friend. The mean age of the donors was 53.0 ± 7.0 years. IVF treatments were done before transplantation. The donor surgery involved uterine isolation with pedicles of the uterine arteries and veins and including large parts of the internal iliacs.

In the recipient bilateral end-to-side anastomosis was accomplished between the uterine artery and one major uterine vein on each side. None needed perioperative blood transfusion and the hospital stays were 3–9 days. The recipients received two ATG treatments perioperatively and corticosteroids for 4 days. They were then only on double immunosuppression with tacrolimus and MMF and the plan was tapered doses of tacrolimus and omission of MMF after 6 months, to avoid possible teratogenic effects of MMF.

Two patients had to be hysterectomized during the initial months due to uterine complications. The other seven patients have shown regular menstruations from 2 months after UTx. The first live birth after UTx occurred in September 2014, when a baby was delivered by c-section in week $31+5$ because of maternal preeclampsia (PE) development. Since then two more births have taken place and these mothers did not develop PE. A fourth recipient is expected to deliver in July 2015.

The four successful pregnancies after UTx are proofs-of-concept of UTx as an effective method to treat uterine factor infertility.

DOI: 10.1530/repabs.2.S006

S007

Mechanical injury of the endometrium to enhance implantation: mechanism of action

Nava Dekel
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Acquisition of uterine receptivity, an essential prelude for successful embryo implantation, is fully dependent on the development of adequate conditions for the attachment of the conceptus to the endometrial epithelium. The particular constituents of such 'adequate conditions' are not as yet defined and markers for a receptive endometrium are practically unavailable. Furthermore, the disappointing, poor rate of pregnancy, presently achieved following the transfer of high quality embryos makes implantation the rate-limiting step for the success of IVF. A substantial increase in pregnancy rate, induced by endometrial biopsyin patients with recurrent implantation failure, has been reported by us and confirmed by others. Along this line, we have later demonstrated that uterine dendritic cells (DCs) are crucial for implantation in mice. Taking these findings into account we raised the hypothesis that local injury generated by endometrial biopsy increases uterine receptivity by provoking inflammation. The overall goal of our study was to unveil the role of inflammation in successful implantation, further providing valuable clinical information that will be translated into diagnosis and treatment of infertility. Our experiments were specifically directed at i) characterization of the response of human endometrial cells to inflammatory-inducing agents, ii) examination of the effect of immune cells on endometrial cell differentiation, and iii) establishment of biomarkers for predicting implantation competence. The results of our study suggest that endometrial biopsy upregulates the expression of proinflammatory cytokines that recruit monocytes to the site of injury and may switch their differentiation into DC-like cells. These monocyte-derived DCs may trigger the epithelial cells to produce molecules that interact with the blastocyst, facilitating implantation.

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SRF New Investigator Award lecture

S008

Molecular mechanisms regulating early equine placental development in health and disease

A M de Mestre, J E Read, B Rose & V Cabrera-Sharp

Early pregnancy loss (EPL) affecting between 6 and 15% of equine pregnancies remains a significant issue for the £3.5 billion Thoroughbred breeding and equestrian industries. Very little is known about the causes of pregnancy failure in the horse, although it is likely to involve a multitude of failed physiological processes and/or environmental insults. Our laboratory aims to identify key mechanisms that regulate the differentiation and function of trophoblast cells in both physiologically normal pregnancies and those that fail. The chorionic girdle is a unique component of the equine conceptus comprised of trophoblast cells that give rise to the equine chorionic gonadotrophin (eCG) producing endometrial cups. The molecular mechanisms that regulate differentiation of chorionic girdle trophoblast and eCG expression remain poorly understood. We have been using a targeted approach to determine the role of bone morphogenetic protein signalling and glial cells missing 1 (GCM1) in regulation of trophoblast differentiation and eCGβ transcription. We have complemented this work with microarray studies that aim to identify novel signalling pathways and candidate transcription factors that may play a role in initiation of these two processes. In order to extend our work to investigate the functionality of trophoblast cells from failed early pregnancy, we have successfully developed new methods to obtain, isolate and culture trophoblast cells from clinical cases of early pregnancy loss. We are currently using this material to determine the incidence and characteristics of aneuploidies associated with the condition.

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SSR New Investigator Lecture

S009

Epithelial genes in the endometrial and ovarian cancer

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One of our major goals is to identify therapeutics and translate our finding to the clinical setting. Over the past few years, we have been focusing on two epithelial specific genes, *CDH1* and *WNT7A*. CDH1 is a transmembrane glycoprotein, belonging to the cadherin superfamily of cell adhesion molecules. When we conditionally delete *Cdh1* in the mouse uterus, infertility results due to disorganized cellular structure of the epithelium and ablation of endometrial glands. However, loss of *Cdh1* alone in the uterus does not predispose mice to tumor formation. Mice with conditional ablation of *Cdh1* and *Trp53* demonstrate architectural features characteristic of type II endometrial carcinomas. In recent studies, we also found that absence of CDH1 and TP53 in endometrial cells initiates chronic inflammation, promotes tumor microenvironment following the recruitment of macrophages, and leads to aggressive endometrial carcinomas. In a second line of investigation, we have revealed a role for the specific WNT ligand WNT7A in epithelial ovarian cancer growth and progression. Our recent studies showed that FGF1 is an oncogenic, direct downstream target of WNT7A/β-catenin signaling, and the WNT7A/β-catenin–FGF1 signaling pathway has potential as a therapeutic target in ovarian cancer. These studies have been supported by NIH/NICHD HD0588222, NIH/NCI CA179214, and ACS-IL 139038.

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SRF Distinguished Scientist Lecture

S010

Reproduction as the pivot for adaptability of the next generation(s), and its health consequences

Richard Sharpe
University of Edinburgh, Edinburgh, UK.

Alterations in the fetal environment can alter lifetime risk of certain diseases. This also applies to reproductive function/disorders in adulthood, and we have shown that fetal androgens play a pivotal time-specific role in this regard in males.
One interpretation of the 'fetal reprogramming' changes that can result in adult metabolic dysfunction is that it represents an adaptive change that better fits the fetus for life after birth. As our evolution has been geared primarily for reproductive fitness, and as the germ cells undergo extensive epigenetic remodeling in fetal life in both sexes, it raises the question of whether 'fetal reprogramming of the germ cells' might also occur in response to an altered fetal environment. In this case, however, any consequences of such reprogramming are not seen in the exposed fetus when it matures but in the offspring that may arise from the 'altered' germ cells. The evidence for such inter-generational effects is growing steadily, but is largely unexplored.
In searching for fetal causes of adult male reproductive disorders, we discovered that paracetamol exposure at therapeutic levels inhibits testosterone production by the fetal human testis. Unexpectedly, we discovered that paracetamol and other analgesics also target the fetal germ cells in both sexes (rats), altering their numbers and expression of the germ cell 'epigenetic machinery'. Consequently, offspring derived from these 'affected' germ cells show an altered reproductive phenotype. This talk will use this and other examples (e.g. dietary manipulation) to illustrate how 'fetal reprogramming' may extend also to the germ cells/reproduction.

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Symposia 3: Management of livestock fertility

S011

Nutritional impacts on fetal development: examples from ovine adolescent models

Jacqueline Wallace
Rowett Institute of Nutrition and Health, Aberdeen, UK.

For ruminant livestock producers, appropriate maternal nutrition during pregnancy is central to the production of viable offspring of optimal birth weight, and impacts postnatal growth, body composition, and reproductive potential. Young still-growing adolescent mothers are particularly vulnerable to nutritional extremes and assisted conception procedures have helped define the relative importance of diverse nutritional exposures. Pre-conception nutrition (via manipulation of embryo donors) does not affect the feto-placental growth trajectory but low maternal nutrient status at conception (via manipulation of embryo recipient BMI) negatively influences placental growth and impacts lamb birth weight. However, it is gestational intake after conception which has the most profound influence on fetal development. In adolescents overnourished throughout gestation (2× optimal control (C) dietary intakes), growth and adiposity of the mother is promoted at the expense of the conceptus. Placental growth, uteroplacental blood flows and fetal nutrient supply are severely compromised and premature delivery of low birth weight lambs (30–40% smaller than C) ensues. A more modest effect on fetal growth (15–17% decrease) is evident in undernourished mothers (0.7×C intake). Here gradual depletion of maternal body reserves directly lowers nutrient availability in the maternal circulation independent of placental size and gestation length is normal. These variations in prenatal nutrient supply differentially impact the structure and function of tissues and organs central to traits of economic importance in postnatal life. Some effects are permanent and sex-specific (e.g. reduced ovarian follicle reserve), while others are transient and/or modified by subsequent nutritional exposures (e.g. hypothalamic-appetite pathways, growth-adiposity trajectories).

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S012

Impact of prenatal stress on reproductive development in livestock

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Many studies demonstrating that the environment a pregnant female experiences can have profound and sometimes persistent effects on offspring development use extreme experimental perturbations which do not reflect the range of environments a pregnant female is actually likely to experience. We have assessed offspring reproductive development following treatments designed to reflect husbandry and management conditions that pregnant sheep or pigs encounter. Female offspring of sows that were mixed with unfamiliar older sows for two, 1-week periods during mid-pregnancy had fewer primordial ovarian follicles, while male offspring had lower oestradiol and testosterone concentrations. Provision of an enriched environment during the first month of life to prenatally stressed male piglets did not alter their reproductive hormone concentrations, suggesting that post-natal treatments do not always modify prenatal effects. Our sheep studies compared offspring of ewes reared in conditions reflecting the range of husbandry conditions on UK farms. Ewes reared in ways that mimicked poorer husbandry had a high stocking density, more competition for food and were mixed. Those managed in line with best practice had lower stocking density, longer feed troughs and remained in stable groups. At puberty, male lambs born to ewes managed in the poorer environment had heavier and larger testes with larger seminiferous tubules and tended to have greater LH receptor gene expression, more Sertoli cells and fewer Leydig cells compared to contemporary male lambs carried by mothers reared according to best practice. These studies suggest that the range of husbandry conditions in current farming systems is sufficient to program reproductive development.

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S013

Embryo-uterine dialogue in the cow

Olivier Sandra
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Successful pregnancy depends on a succession of complex biological processes that are regulated temporally and spatially. In term of contribution to pregnancy, the mother not only produces oocytes but she also hosts the whole gestation, mainly in the uterus. For decades, a major focus has been made on oocyte and embryo quality in term of contribution to progression and issue of pregnancy in mammals, including cattle. Nevertheless, in the bovine species, recent studies have clearly established the impact of uterus quality on pregnancy issue as early as interactions with the conceptus take place. In dairy and meat cattle, persistent or transient modifications in organisation and functionality of the endometrium before and during reproductive life (e. g. nutrition, infections) can dramatically affect pre-implantation embryo trajectory through epigenetic alterations, with lasting consequences on subsequent progression of pregnancy as well as post-natal health. In addition to this *driving* property of the endometrium, distinct endometrial responses can also be elicited by bovine embryos presenting distinct post-implantation fates, e.g. when they are generated by artificial insemination, IVF or by somatic cell nuclear transfer. Molecular and cellular data will be presented as illustrations of the driver-sensor properties of the bovine endometrium. Evaluation of endometrial quality and restoration of the endometrial physiology to a level compatible with term pregnancy represent two ways that can be used to increase fertility in cattle, provided that applied treatments are devoid of detrimental effects on expression of production traits in adult.

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Oral Communications

SRF Post Doctoral Prize Session

O001

Hyperandrogenism modulates adipokine gene expression in mouse adipocytes: implications for PCOS

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Imperial College London, London, UK.

Polycystic ovary syndrome (PCOS) is a common endocrinopathy that is associated with anovulatory infertility, menstrual disturbances as well as an adverse metabolic profile. Hyperandrogenism is the hallmark of PCOS and originates predominantly from ovarian theca cells. Obesity increases androgen synthesis, partly due to accompanying hyperinsulinemia but also as a result of an effect of adipokines on ovarian steroidogenesis (Comim *et al*. *PLoS ONE* **8**, 2013). Adipokines are factors secreted by adipose tissue and while adipokines are known to increase androgen synthesis, the effect of hyperandrogenism on adipokines is less clear. In this study, we investigated the effect of excess androgens on adipocyte differentiation and adipokine gene expression. Immortalised multi-depot mouse preadipocytes were differentiated for 14 days in the presence and absence of dihydrotestosterone (DHT). In addition, fully differentiated adipocytes were treated with DHT or control for 24 h. Adipogenesis was observed using Oil Red O and quantitative PCR was used to analyse gene expression for a panel of 25 adipokines. Our results show that hyperandrogenism leads to dysregulation of adipokine gene expression. Several adipokines thought to be involved in modulating ovarian androgen secretion are significantly perturbed. These include downregulation of adiponectin ($P<0.05$) and upregulation of visfatin ($P<0.05$). Other changes include distinctive adipokine profiles similar to those found in metabolic syndrome such raised PAI1 ($P<0.05$) as well as a downregulation of adipokines related to energy expenditure and brown adipose tissue identity. These results support the existence of a reciprocal relationship between hyperandrogenism and adipokine secretion.

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O002

Hyperphagia of pregnancy and lactation is associated with changes in appetite-regulating gut hormones and gastrointestinal modifications in Wistar rats

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Introduction
Pregnancy and lactation result in increasing maternal appetite and adiposity, which in humans may lead to long-term weight retention. Previous studies in this area are limited, but some suggest that the appetite-inhibiting (anorexigenic) gut hormone peptide-YY (PYY) is increased in lactation, despite hyperphagia. This work characterised changes in orexigenic (appetite-stimulating) ghrelin and anorexigenic PYY and glucagon-like peptide 1 (GLP1) and gut architecture during pregnancy and lactation.
Methods
Female Wistar rats, kept under reverse lighting (lights off 1100–2300 h), were sampled during the dark phase at pregnancy days 4, 12, and 18 and lactation days 0, 5, 10, and 25. Peptides were measured in matched fed and fasted plasma and in gut tissue using radioimmunoassay. Detailed gut measurements were standardised by free-floating tissue and maximal relaxation with nicardipine and were used to determine how gut architecture may change in relation to enteroendocrine cell density and/or peptide concentration. Enteroendocrine cells were quantified using immunofluorescence.
Results and discussion
Fasted plasma ghrelin during pregnancy was significantly ($F(2,18)=3.767$, $P=0.043$) highest in day 4 pregnant dams and significantly ($F(3,24)=4.546$, $P=0.012$) increased by day 25 of lactation. Ghrelin-immunoreactive stomach cells were significantly ($F(2,17)=29.735$, $P<0.001$) increased at day 0 of lactation (d0L) compared with day 12 pregnant and proestrus controls, and stomach tissue ghrelin concentration was also significantly (Kruskal–Wallis, $\chi^2=10.057$, df = 3, $P=0.018$) increased at d0L. These results suggest that increased ghrelin supported the onset of lactation-associated hyperphagia. Significantly increased GLP1 and PYY levels in colon tissue during early lactation were associated with significantly increased gastrointestinal size at this time, not satiety. GLP1 in fed plasma ($F(3,21)=5.505$, $P=0.006$) and both ascending colon PYY ($F(3,22)=4.638$, $P=0.012$) and GLP1 ($F(3,22)=4.164$, $P=0.018$) levels were significantly reduced in late lactation, also supportive of the marked hyperphagia of late lactation by a reduction in satiety.

DOI: 10.1530/repabs.2.O002

O003

Kinetic, morphological, and functional details of the early stages of human and mouse embryo implantation in an *in vitro* model

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A significant proportion of IVF treatments are unsuccessful due to defects in implantation. The first stage of implantation is an attachment interaction between the trophectoderm (TE) of the blastocyst-stage embryo and the uterine luminal epithelium (LE). Endocrine signalling from the corpus luteum and paracrine signalling from uterine glands, uterine stroma and the embryo promotes LE receptivity. To investigate the LE–TE interaction we have developed an *in vitro* model using the human endometrial adenocarcinoma Ishikawa cell line with human and mouse blastocysts. From a cohort of >100, we observed stable attachment of hatched day 6 human blastocysts to Ishikawa cells within 18 h. Immunostaining of attached human blastocysts at 48 h revealed distinct stages of implantation; apoptosis subjacent to blastocysts at the early stages of breaching the LE, TE syncytialisation at points of attachment to LE, and extensive TE syncytialisation at multiple sites of the outgrowing blastocyst after breaching the LE. Hatched mouse blastocysts were competent for stable attachment on day 6 and detailed kinetic analysis demonstrated initial attachments progressed to breaching the LE and TE outgrowth within 24 h. 12/46 attached mouse blastocysts achieved this in ≤ 12 h. Immunostaining weakly and stably attached mouse blastocysts revealed changes in TE and LE morphology, LE apoptosis and the apical localisation of candidate TE-LE attachment proteins such as integrin $\alpha v\beta 3$, CD44, and osteopontin. Together these data evidence mechanisms of blastocyst attachment to and progression beyond the LE, and validate this *in vitro* model as a tool to develop treatments for implantation failure.

DOI: 10.1530/repabs.2.O003

Oral Communications 1: Embryo and Implantation

O004

The bioenergetic profile of the bovine blastocyst

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The preimplantation embryo undergoes dynamic changes in energy demand during development. Oxygen consumption rate (OCR), representing overall oxidative metabolism, has been reported in several species but few studies have examined the components of OCR. Individual blastocyst OCR was measured using a non-invasive oxygen probe (unisense), whereas OCR of grouped blastocysts was determined using the Oxygen Biosensor fluorometric assay (BD Bioscience). Respiratory chain inhibitors were used to assess different components of OCR. Data was analysed using ANOVA with *post-hoc* Bonferroni's test. Addition of 0.2 μM antimycin to inhibit mitochondrial OCR caused mean OCR to fall from 26 ± 4.7 to 2.3 ± 1.3 pmol/embryo per h, indicating that ~87% of OCR was accounted for by mitochondrial activity. Inhibiting ATP synthase with 0.05 μg/ml oligomycin reduced mean OCR from 19.7 ± 2.8 to 6.4 ± 2.3 pmol/embryo per h, meaning that 33% of OCR was uncoupled from ATP synthesis. Addition of the uncoupler 2,4-DNP increased mean OCR from 17.1 ± 2.5 to 32.3 ± 2.7 pmol/embryo per h ($P\leq0.001$). The difference between these values, known as the spare respiratory capacity, was 15.2 pmol/embryo per h. Following inhibition of complex I with 0.01 μM rotenone, mean OCR fell from 27.7 ± 1.6 to 11.6 ± 0.9 pmol/embryo per h, suggesting that 16.1 ± 1.1 pmol/embryo per h was complex I-dependent. Overall, 66% of basal blastocyst OCR was coupled to ATP synthesis, with 58% driven by the NADH-dependent complex I and 8% by the $FADH_2$-dependent complex II. 21% of basal OCR was uncoupled while non-mitochondrial processes accounted for 13%. A +89% 'spare' capacity may enable ATP synthesis to increase in response to high energy demand processes e.g. blastocoel expansion. This profile highlights plasticity in metabolic regulation and allows re-interpretation of existing data.

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O005

Effect of environmental oxygen on the expression of miRNAs in human embryonic stem cells

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Human embryonic stem cells (hESCs) are derived from the inner cell mass of the blastocyst. They proliferate by self-renewal and have the potential to develop into all cells of the three germ layers. Thus, hESCs hold great potential for use in regenerative medicine. hESCs are difficult to maintain in culture and have a tendency to spontaneously differentiate. Culture at a low, 5% oxygen concentration is beneficial for the maintenance of a wholly pluripotent cell population compared to atmospheric oxygen but the mechanism which regulates this hypoxic response is not known. We hypothesise that culture under hypoxic conditions alters the expression of microRNAs (miRNAs) to promote stem cell maintenance. Three low-density miRNA arrays were performed on hESCs cultured at either 5 or 20% oxygen to investigate differentially expressed miRNAs. In total, 231 miRNAs were expressed in hESCs and 40 miRNAs were found to be differentially expressed at 5% oxygen compared to 20% oxygen. miR-210 was found to be consistently upregulated in hESCs cultured at hypoxic conditions compared to atmospheric oxygen. This finding was validated using qPCR and miR-210 expression was found to be fourfold greater ($P<0.05$) in Hues7 hESCs cultured at 5% oxygen compared to 20% oxygen. Bioinformatic and in silico analyses on differentially expressed miRNAs are being used to predict those which may target key genes regulating stem cell fate. These data suggest that environmental oxygen tension regulates the expression of miRNAs in hESCs.

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O006

Mechanism of microRNA lethal-7 in embryo dormancy in mice

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Embryo dormancy (embryo diapause) is a reproductive strategy used by ~100 different mammals to avoid the risk of exposing their offsprings to unfavourable environmental conditions by delaying embryo implantation. The blastocysts from domestic sheep, rabbit or cattle enter into diapause after being transfer into mouse uteri induced to have delayed implantation. The observation suggests that blastocysts from all mammals, including that of human, may have an ability to enter into diapause. The molecular mechanism of embryonic diapause in all species is not yet clear. Our previous data showed that the levels of let-7 were relatively high in diapause embryos compared to reactivated embryos by E_2 in mice. The embryos electroporated with let-7 can still be alive even after the culture for up to day 13. Delayed implantation model showed that the activated embryos changed into diapause state if these embryos were electroporated with let-7. Importantly, 12.5% of diapause embryos induced by let-7 *in vivo* developed to term after culture for 4 days (i.e. day 8). Microarray results showed that gene expression profiles were similar between let-7-treated and P_4-induced dormant embryos compared to D4 or E_2-activated embryos. Further analysis demonstrated that cell cycle and metabolism related genes were involved in the roles of let-7 in induction of embryo dormancy. In conclusion, let-7 has an important role in the induction of embryo dormancy in mice.

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O007

A potential co-culture system of ovine blastocyst with uterine endometrium to mimic initial attachment of embryo implantation

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In livestock ruminant species including sheep and cattle, high fertilisation rates shown around 80%, do not necessarily equate to successful pregnancy. Deficient uterine function is therefore a major contributory factor to pregnancy failure resulting in embryonic mortality. In humans undergoing IVF treatment for subfertility, approximately only 25% of embryo transfers will successfully implant. To gain a better understanding of the natural implantation environment, *in vitro* embryo-endometrium models have the potential to overcome studying inaccessible implantation sites *in vivo,* and investigate factors required for embryo attachment.

The aim of this study was to establish an ovine three-dimensional (3D) co-culture implantation model, to assess blastocyst attachment, and provide a functionally viable model for future study of specific aspects of implantation. In this preliminary study, both uteri and ovaries were collected from a local abattoir. Endometrial primary cell cultures were constructed from epithelial monolayers grown on collagen cell inserts, co-cultured with monolayers of stromal cells to maintain endometrial epithelial–stromal architecture. IVF was performed on aspirated oocytes, and embryos were cultured to blastocyst stage (days 6–8), simultaneous to the endometrial culture, steroid treated to induce uterine receptivity. Co-cultures were constructed using pooled blastocysts randomly allocated to microwells containing cell inserts of confluent epithelial/stromal constructs for 48 h.

High percentages (82%) of transferred embryos retained their original position and were confirmed attached at respective implantation sites, following agitation of microplates and some lateral outgrowth of trophoblast cells was observed. This model has the distinct advantage of using primary cells as opposed to cell lines, which maintain functional characteristics and are hormone responsive. Preliminary immunofluorescence data detected receptivity biomarkers, integrins and osteopontin on blastocysts and endometrial cells, indicating this model is potentially a future tool for the study of the molecular mechanisms of implantation/failure.

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O008

The effect of IVF and embryo culture on mouse development and adult health

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Several million babies have been born worldwide since IVF and assisted-reproductive technologies (ART) became available. However, reports link IVF techniques with adverse short- and long-term health outcomes.[1,2,3] Using a mouse model, we investigated the effect of IVF on the total cell number in blastocyst stage embryos and the postnatal health of offspring.

Methods

Experimental groups; (NM) C57/BL6 non-superovulated females naturally mated with CBA males. (IVC) Two-cell stage embryos collected from C57/BL6 superovulated females mated with CBA males and transferred to MF1 pseudo pregnant recipients. (IVF) Conducted on isolated C57/BL6 oocytes from superovulated females using CBA sperm following an IVF protocol;[4] embryos at two-cell stage transferred as above. Blastocyst trophectoderm (TE) and inner cell mass (ICM) cell numbers from NM and IVF embryos were determined by differential staining.[5] Offspring were analysed for body weight, systolic blood pressure (SBP).

Results and discussion

NM blastocysts (41 embryos from 12 mothers) had significantly more TE and ICM cells ($P<0.05$) compared with IVF (25 embryos; 12 mothers). Male (26–40; 27 mothers) and female (21–38; 27 mothers) offspring from IVC and IVF embryos were heavier than NM offspring from week 6 and 5 ($P<0.05$); respectively; no differences between IVC and IVF weights were present. IVF and IVC induced increased SBP in male (weeks 9 and 15) and female (week 15) offspring vs NM ($P<0.05$). We conclude IVF causes reduction in blastocyst cell numbers but superovulation and embryo transfer are sufficient to alter offspring growth and SBP. Further offspring growth, SBP and glucose tolerance analyses are underway.

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Oral Communications 2: Ovarian function

O009

Role of KIT ligand in nuclear maturation in bovine oocytes

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Oocytes are arrested in prophase I until the LH surge, which stimulates an EGF-like growth factor signaling cascade critical for cumulus expansion, and releases meiotic arrest while inhibiting the expression of natriuretic peptide C (NPPC). In rodents, there is evidence that KIT ligand (KITLG) secreted from granulosa/cumulus cells may also enhance nuclear maturation of the oocyte. The objective of the present study was to measure the expression of KITLG in bovine cumulus–oocyte-complexes (COCs) during *in vitro* maturation (IVM), and to determine whether KITLG alters nuclear maturation. Bovine ovaries were collected from an abattoir, and COCs were aspirated from follicles 3 to 8 mm diameter and cultured for 22 h in a serum-free IVM medium with FSH and LH. Abundance of *KITLG* mRNA in cumulus cells increased with time during IVM, with a maximum at 12 h. The KITLG receptor, KIT, was expressed in oocytes, but mRNA levels did not change during IVM. Addition of KITLG to COCs for 22 h did not alter cumulus expansion, but significantly increased the proportion of oocytes progressing to metaphase II (54±3% vs 66±3% for 0 and 50 ng/ml KITLG). We then assessed potential mechanisms of action of KITLG. Addition of graded doses of KITLG to COCs significantly inhibited *NPPC* mRNA levels in cumulus cells. KITLG did not alter levels of mRNA encoding oocyte secreted factors (BMP15, GDF9) in the oocyte, but significantly increased abundance of mRNA encoding the maturation-associated protein, Y box binding protein 2 (YBX2). Finally, we assessed the effect of known oocyte-secreted factors on the expression of *KITLG* in cumulus cells; BMP15 and FGF8 increased *KITLG* mRNA levels, whereas FGF17 had no effect. The present study shows that KITLG enhances nuclear maturation of bovine oocytes, and may do so in part by decreasing levels of NPPC in cumulus cells and by increasing YBX2 levels in oocytes.

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O010

Role of Wnt/β-catenin signal transduction pathway in rat follicular development and luteal function

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The evolutionarily conserved Wnt/β-catenin signal transduction pathway controls many biological processes. The objective of this study was to determine the role of this signaling pathway in follicular development and luteal function. To this purpose PMSG–eCG gonadotropin-prepubertal treated female rats were injected with a Wnt inhibitor (XAV939, 5 μg/ovary, Wnt-I group) or vehicle (DMSO, control group) into the bursa of both ovaries the day of hCG administration. Two days after hCG and Wnt-I or vehicle injection, blood samples and ovaries were collected. Ovarian function was evaluated by measuring serum progesterone (P_4) (RIA), steroidogenic-regulators protein levels by western blot, apoptotic parameters and ovarian structures at different stages of development. The levels of P_4 significantly decreased after Wnt inhibitor administration. Corpora lutea (CL) were isolated by microdissection and steroidogenic protein regulators were measured. StAR levels significantly decreased in the Wnt-I group in comparison with the control group, whereas the levels of P450scc or 3β-HSD enzymes did not change. IHC studies showed that luteal cells exhibited high staining for active Caspase 3 in the Wnt-I group in comparison with the control. Histological studies showed a tendency to a decrease in the percentage of CL and an increase in antral follicles in the Wnt-I group. In conclusion, the inhibition of Wnt pathway appears to produce a decrease in P_4 serum levels associated to a decrease in StAR levels in CL, an increase in active Caspase 3 expression and a tendency to inhibit luteinization.

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O011

The role of the oocyte is investigated in premature ovarian failure using the reaggregated ovarian pellet technique

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Premature ovarian failure (POF) affects around 1% of women and is idiopathic in 74–90% of cases. Our mouse model of POF, the double mutant (DM), are fertile at 6-weeks and undergo POF by 3-months consistent with a decline in developing follicles. However, DM ovaries have increased numbers of primary follicles at the 3a stage, indicating a block in follicle development.

We investigated whether we could rescue the ability of DM oocytes at 3- and 9-weeks to coordinate follicle development by replacing DM somatic cells with WT using the reaggregated ovarian pellet (ROP) technique. Production of a ROP involves separation of the ovary into single cells, isolation of the different cell types, and then combining cells to generate a chimeric ROP. The ROP is transplanted under the kidney capsule of ovariectomised immune-compromised mice to develop for 21 days when antral follicles can be observed.

In ROPs generated using 3-week DM oocytes and WT somatic cells ($n=3$), follicles were present from the secondary stage onwards suggesting the DM 3a follicle development block was overcome. However, ROPs generated using 3-week control oocytes contained follicles from all stages but in much higher numbers. For ROPs generated using 9-week old control ($n=3$) or DM oocytes ($n=3$), although follicle numbers were equivalent between control and DM, follicles in the DM ROP were at more advanced stages of follicle development compared to control.

In summary, oocytes from mice with POF retain the potential to regulate follicle development and thus this technique identifies a potential treatment for POF.

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O012

Differential effects of transforming growth factor beta 2 in two different models of preantral follicle growth

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The onset of mammalian follicle development is marked by granulosa cell (GC) proliferation, GC shape change, and oocyte growth. Despite their critical role in female fertility the factors and mechanisms underlying these cellular changes remain largely unknown. The transforming growth factor beta (TGFβ) superfamily has been strongly implicated in early follicle growth. Disruption of the function or expression of members of the TGFβ superfamily, receptors or key components of the downstream SMAD signalling pathway induces profound effects on the number and developmental potential of preantral follicles. This study aimed to examine the role of TGFβ2 on follicle activation and preantral follicle growth.

Postnatal day 4 (PND4) mouse ovaries (C57BL/6) were treated with TGFβ2 (1, 10, and 100 ng/ml) in a whole-ovary culture system. Cultured ovaries were fixed for immunohistochemical localization of vasa and laminin and caspase-3. Image analysis was performed to quantify follicle proportions and abundance of caspase-3 staining. TGFβ2 suppressed follicle activation in the whole ovary as demonstrated by a smaller proportion of growing follicles in TGFβ2 treated ovaries compared to vehicle treated ($n=3$–6 ovaries, logistic regression; 1 ng/ml ($P<0.05$) and 100 ng/ml ($P<0.01$)). No qualitative differences in caspase-3 staining were demonstrated between treatment groups (i.e. no evidence of TGFβ2 toxicity).

Preantral follicles were isolated from PND15 mouse ovaries and cultured with TGFβ2 (0.01, 0.1, and 1 ng/ml) or TGFβ2 neutralizing antibody (100 and 10 ng/ml). Follicle area was measured at 0, 24, 48, and 72 h (ImageJ). TGFβ2 promoted increased growth of isolated follicles, relative to vehicle, at all concentrations after 24, 48, and 72 h ($n=6$ ovaries, two-way ANOVA; $P<0.0001$). The TGFβ2 antibody suppressed the effect of exogenously applied TGFβ2. However, at the doses used, the antibody did not supress follicle growth below that of the vehicle suggesting it is not affecting any endogenous TGFβ2. In conclusion, our results highlight differential effects of TGFβ2 in two different models of preantral follicle growth.

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O013

The oocyte induces estrus stage-specific changes in theca cells and their extracellular matrix

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The oocyte influences hormonal and cellular changes in the follicle and the ovary. Theca cells, surrounding the follicle basement membrane, synthesise androgen and thus regulate ovarian oestrogen production. The vascular theca layer provides nutrients to the avascular inner follicle. In *C1galt1* mutant mice, all oocytes lack core 1-derived *O*-glycans due to oocyte-specific ablation of core 1 beta 1,3-galactosyltransferase. *C1galt1* mutants have increased fertility resulting from modified follicle development and thus endocrinology. Since testosterone levels were decreased in the mutant, we investigated if the quantity of theca cells was modified during follicle development. Ovaries were collected at the four stages of the estrus cycle and subsequently fixed, paraffin embedded, and sectioned. Hyaluronic acid binding protein, which localised to theca cells, was detected using immunohistochemistry. Follicle development was assessed measuring the area and depth of theca cells, and the intensity of hyaluronic acid detected, using ImageJ. Theca area, depth, and hyaluronan levels were decreased in mutant primary (stages 3a and 3b) and secondary (stage 4) follicles compared to controls at metestrus, whereas at diestrus only theca depth was decreased. Mutant preantral follicles (stages 5a and 5b) also exhibited a decrease in theca area and depth and thus follicle area compared to control at metestrus. Theca cells of mutant antral follicles (stages 5a+A and 5b+A) contained less hyaluronan compared to control follicles at metestrus. Large antral mutant follicles had a reduced theca area at metestrus compared to controls but increased hyaluronan levels at proestrus. Overall, mutant follicles have less theca and hyaluronan in theca extracellular matrix, most evident at the metestrus stage. These results highlight the role of the oocyte in regulating both the amount of theca cells and extracellular matrix. They also demonstrate the rapid and dynamic changes that occur throughout the estrus cycle, with theca cell structure changing on a daily basis.

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SRF Student Prize Session

O014

Maternal protein restriction around conception reduces the neural stem cells during mouse fetal brain development and alters neuronal differentiation during gestation

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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. 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). 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. We have shown using *in vivo* and *in vitro* techniques, 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 (E14.5 and E17.5). Moreover, Emb-LPD causes remaining NSCs to upregulate the neuronal differentiation rate in compensation beyond control levels. This data demonstrates poor maternal nutrition around conception, already associated with adult behavioural deficit, adversely affects early brain development.

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O015

Defective decidualisation: a possible mechanism of *Chlamydia trachomatis* induced miscarriage

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Miscarriage affects one in five pregnancies and has serious physical and psychological implications for the patient. Maternal infections account for 15% of miscarriages. *Chlamydia trachomatis* has been associated with miscarriage however the epidemiological data to support this are conflicting. The mechanism explaining the association is also unknown. Our objective was to determine whether *C. trachomatis* infection leads to miscarriage by impacting upon endometrial decidualisation, a process crucial for successful embryo implantation and early pregnancy.

We developed a novel *in vitro* model of *C. trachomatis* infection and decidualised primary endometrial stromal cells. Cells exposed to progesterone and cAMP were decidualised *in vitro*, and were subsequently infected with *C. trachomatis* serovar E. U.v.-inactivated *C. trachomatis* and uninfected cells were used as controls. Gene of interest changes were measured both at mRNA and protein level using RT-PCR and ELISA respectively.

We demonstrated that *C. trachomatis* can infect and multiply in endometrial stromal cells. *C. trachomatis* positive inclusions containing high numbers of bacteria were detected in infected cells compared to controls 48 h post infection ($>1\,000\,000$ plasmid copies, $P<0.01$, $n=4$). Classic decidualisation marker prolactin protein levels were lower in decidualised infected cell supernatants compared to control cells ($n=4$, $P<0.05$). *C. trachomatis* infection induced an innate immune system response from stromal cells, as measured by CXCL8 secretion, a chemokine that attracts neutrophils ($n=5$, $P<0.05$). On the contrary, chemokines CXCL12 and CXCL16, which are known to be essential for the invasive capability of trophoblast cells, were reduced in infected decidualised cells ($P<0.05$, $n=4$ and $P<0.01$, $n=5$ respectively).

Our data suggest that *C. trachomatis* infection of the endometrial stromal cell compartment can result in impaired decidualisation, because markers such as prolactin, CXCL12 and CXCL16, are reduced to levels similar to those detected in non-decidualised stromal cells. This mechanism could explain the role of *C. trachomatis* infection in adverse pregnancy outcomes.

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O016

Investigating a role for the epidermal growth factor receptor in androgen signalling within mouse preantral follicle development

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Hyperandrogenism and dysregulated follicle development both characterise polycystic ovary syndrome (PCOS), however little is known about how androgens affect early preantral follicle development. Recent evidence suggests that androgens may act, in part, through non-classical modulation of growth factor signalling pathways. Members of the epidermal growth factor (EGF) family play a role in promoting preantral follicle development in the mouse, with receptor subtypes EGFR, ErbB2, and ErbB3 detected in primary and secondary follicles. This study aimed to investigate androgen–EGF interaction within preantral follicles.

Preantral follicles obtained from PND16 mouse ovaries (C57BL/6) were mechanically isolated and cultured in the presence of EGF (10 ng/ml) or dihydrotestosterone (DHT; 10 nM) with or without the specific EGFR inhibitor AG1478 (10 mM). Granulosa cells were isolated from PND26 mice (C57BL/6) and cultured with DHT (10 nM).

DHT increased preantral follicle growth from 24 h onwards ($P<0.05$). Combined treatment with EGF and DHT resulted in elevated growth above that of either treatment alone ($P<0.05$). DHT treated follicles displayed no change in EGFR, ErbB2, or ErbB3 mRNA, however, ErbB2 protein expression was down-regulated at 24 h ($P<0.001$), suggesting a role for androgens in regulating ErbB abundance, independent of transcription. The addition of AG1478 not only ablated EGF stimulated follicle growth but also attenuated the effect of DHT on growth ($P<0.01$) implying that the actions of DHT are mediated, at least in part, through the EGFR. Granulosa cells treated with DHT, showed a significant increase in phospho-ERK at 2 and 5 min ($P<0.05$), indicating the presence of non-genomic androgen signalling within mouse granulosa cells. Whether this non-classical pathway involves the EGFR is unclear and is currently under investigation. In conclusion, DHT stimulated follicle growth is mediated partly

through the EGFR, likely through both genomic and non-genomic signalling in the preantral follicle.

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O017

Decidual macrophages become increasingly immunosuppressive during early pregnancy and an altered activation state may be associated with a higher risk of preeclampsia

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Human pregnancy represents a unique immunological environment. However, very little is known about the role of uterine decidual macrophages (dMs) in early human pregnancy. The monocytic cell line (THP1) was activated and polarised to a pro-inflammatory M1 or immunosuppressive M2 M phenotype and the conditioned media was used to treat fetally-derived trophoblast cells. Treatment with M1 conditioned media was found to significantly reduce trophoblast motility *in vitro*, indicating that M phenotype may have a role in regulating placentation. Primary dMs were isolated from tissue obtained from women undergoing first-trimester terminations of pregnancy. Receptor profiling by flow cytometry demonstrated that dMs have a unique expression profile with expression of both M1 and M2 markers of polarisation. However, they become significantly more M2-like in phenotype as pregnancy progresses, with an increase in CD206 expression and down-regulation of activation markers such as HLA-DR and CD86, between 4 and 14 weeks gestation. In addition, the relative risk for developing preeclampsia was calculated by uterine artery resistance indices (RI) measured by Doppler ultrasound prior to pregnancy termination. DMs isolated from pregnancies with a higher risk of developing preeclampsia (high-RI) were found to have an altered phenotype when compared to Ms from normal risk pregnancies (normal-RI). In conclusion, M polarisation state has been shown to affect macrophage-trophoblast interactions *in vitro*. Furthermore, there are dynamic changes in dM phenotype during early pregnancy and aberrant dM activation may contribute to a higher risk of developing preeclampsia.

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O018

Variations in the way UK pregnant sheep are managed programs male reproductive development

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This study determined whether variation (representative of normal UK farm conditions) in pregnant ewe management is sufficient to alter the programing of the male reproductive axis. Between days 85 and 138 of pregnancy, twin-bearing ewes were managed to either mimic 'poor husbandry' (PH) with a high stocking density, reduced feeding space and repeated social mixing or according to 'best practice' (BP) with a lower stocking density, longer feed troughs, and stable social groups. Testes were collected from male offspring at 7 (pre-pubertal, $n=10$/prenatal treatment) and 19 weeks (puberty, $n=8$/prenatal treatment) of age, weighed and preserved for histology and gene expression analyses. Proliferation of testes cells was determined by immunohistochemical localisation of Ki-67 and counterstaining with haematoxylin. There was no treatment effect on lamb body weight at either age or on 7-week-old lamb testis weight or seminiferous tubule diameter. Testes from pubertal PH lambs were heavier (352 ± 16 g vs 257 ± 16 g; $P=0.002$), with a larger volume ($39\,219\pm3293$ mm^3 vs $29\,498\pm2092$ mm^3; $P=0.015$) and increased seminiferous tubule diameter (Feret's diameter $=272.36\pm8.81$ μm vs 246.81 ± 5.24 μm; $P=0.015$) compared with BP lamb testes. Testes from BP lambs had more proliferating Leydig cells than PH lambs at both ages (7 weeks: 98 ± 28 vs 35 ± 20 stained cells/mm^2 and 19 weeks: 34 ± 15 vs 18 ± 4 stained cells/mm^2, respectively; $P=0.025$). PH lamb testes tended to have greater LH receptor expression, more Sertoli cells but fewer Leydig cells compared to contemporary BP lambs. These studies suggest that the range of husbandry conditions in current farming systems is sufficient to program male reproductive development.

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O019

Obesity in PCOS: a consequence of prenatally programmed reduced energy expenditure

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Polycystic ovary syndrome, a common endocrine condition affecting up to 10% of women of reproductive age, is associated with an increased risk of developing insulin resistance and obesity. Obesity is associated with increased energy intake or reduced energy expenditure. In this context, postprandial thermogenesis (PPT), is an important constituent of energy expenditure.

Our lab utilizes a clinically realistic ovine model of PCOS, where pregnant Scottish Greyface ewes are treated biweekly with either 100 mg of testosterone propionate (TP) or vehicle control (C) from days 62 to 102 of gestation. We measured PPT in adult female offspring ($C=11$; $TP=4$) through implantation of datalogger thermometers into subcutaneous fat. Glucose tolerance tests were a second experimental readout.

Prenatally androgenized female sheep had normal birthweight and postnatal growth to adolescence. However, as adults (2.5 years old), TP-exposed animals had increased body weight ($P<0.05$), increased fasting insulin concentrations ($P<0.05$), and decreased fasting glucose to insulin ratios ($P<0.05$). TP exposed animals showed decreased peak postprandial increase in temperature ($P<0.05$) but this was not primarily a function of increased body weight as a difference in PPT was also observed between a subset of matched obese controls and TP exposed animals. Prenatally androgenized females also demonstrated increased latency from commencement of feeding to maximal postprandial temperature ($P<0.05$) when compared with controls. The reduction in PPT and increased time to reach maximal temperature post-feeding correlated positively with increased levels of fasting insulin ($r=0.56$; $P<0.05$ and $r=0.68$; $P<0.05$ respectively).

Prenatally androgenized female sheep are destined to have increased body weight most likely due to a reduction in the capacity for energy expenditure, which is mirrored in women with PCOS. This gives us a unique opportunity to investigate the molecular regulation of PPT to develop new interventions to target obesity in PCOS.

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Oral Communications 3: Sperm

O020

Sertoli-specific ablation of RHOX8 results in impaired spermatogenesis in mice

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The reproductive homeobox X-linked, *Rhox*, genes encode transcription factors that are expressed selectively in reproductive tissues. While there are 33 testis-expressed *Rhox* genes in mice, only *Rhox5* and *Rhox8* are produced by Sertoli cells, suggesting that they alone regulate somatic-cell gene products crucial for germ-cell development. We used the *Rhox5* promoter to drive tissue-specific RNAi to knockdown (KD) RHOX8 *in vivo*. Western and immunohistochemical analysis confirmed Sertoli-specific KD of RHOX8. However, other Sertoli markers, *Gata1*, *Ar*, and *Rhox5*; maintained normal expression patterns, suggesting the KD affect was specific. Male RHOX8-KD animals showed reduced fecundity in timed breeding experiments, ~50% decline in spermatogenic output, and 30% decrease in sperm motility, in four independent *Rhox8*-KD lines. Increased germ cell apoptosis and a delay in spermatogenesis stages VI–VII transition contributed to the phenotype. Analysis of established RHOX5-regulated genes found some apoptosis related factors similarly misregulated in RHOX8-KD mice. However, *Rhox7*, *Rhox10*, and *Rhox11* were uniquely downregulated in RHOX8-KD testes. At present the function of these genes in germ cells is unknown. In postnatal Sertoli cells, *Sox8* (partially) and *Sox9* (strongly) were downregulated in RHOX8-KD testes. These genes are essential factors for Sertoli development and function and are likely key mediators of RHOX8 action for maximal sperm production. We are currently investigating this hypothesis, as well as other genetic interactions; through the characterization of a

conditional Cre-LoxP activated shRNA KD model that can be employed to elucidate *Rhox8*'s role in embryonic testis development as well as confirm our present postnatal results.

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O021

Ruminal acidosis has long-term effects upon sperm production in the bull

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Seedstock bulls in Australia are commonly fed high-energy supplements prior to sale. These supplements contain readily fermentable carbohydrates, which may precipitate ruminal acidosis. We hypothesise that a single transient acidotic SARA event would decrease semen production sufficient to preclude bulls from BBSE thresholds and thereby sale.

Santa Gertrudis bulls ($n=10$, age 20 ± 6 months) were fed in yards on *ad lib* hay with daily individual grain feeding prior to acidosis challenge. Semen was collected fortnightly prior to challenge with sperm parameters and blood hormone parameters determined. Ruminal acidosis challenge treatments consisted of either a single oral dose of oligofructose (6.5 g/kg LW) or an equivalent dose of water in bulls that had achieved a stable spermiogram. Rectal and scrotal temperature, heart rate, rumen pH and tone, were monitored during challenge with concomitant ruminal fluid collection. Ejaculates and blood were collected every 3rd day for a period of 7 weeks, then once weekly until 3 months post-challenge.

Oligofructose treatment decreased rumen pH from 2 to 16 h post treatment ($P\leq0.006$) with associated increases in individual VFAs, D-lactate, ammonia and respiration rate. Measures of sperm quality decreased in oligofructose treated bulls from 18 days post treatment and remained significantly lower upon completion of the study at 90 days post challenge ($P=0.02$). This was associated with increased cortisol and decreased FSH and testosterone in these bulls ($P\leq0.05$).

This is the first study to show that a transient acidosis event is sufficient to induce effects upon sperm production in bulls for up to 3 months, thereby supporting our hypothesis. Oligofructose was shown to be effective in inducing a SARA event where a compact dosing form is required in unhandled range bulls. In the collected ejaculates, as anticipated, there was a transition through the recognised sequence of sperm abnormalities, according to stage of spermatogenic cycle or position in tract, when the acidotic event occurred.

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O022

Interaction of progesterone and pH in regulation of hyperactivated motility in human spermatozoa

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Catsper is a Ca^{2+} permeable ion channel in the principal piece of the sperm flagellum that is crucial in regulation of motility. It is multimodally sensitive, being activated by voltage, pHi and diverse agonists including progesterone. Elevation of pHi and progesterone both shift voltage-sensitivity of the channel to more negative potentials and thus may act synergistically to enhance Ca^{2+}-influx.[1] However, intracellular alkalinisation of human sperm with NH_4Cl did not enhance the $[Ca^{2+}]_i$ response to progesterone.[2] We have investigated the interaction of the effects of progesterone and extracellular pH (pH 7.4 and 8.5) on hyperactivaton of human sperm, assessed using CASA (Hamilton-Thorne CEROS). At pH 7.4, progesterone increased sperm velocity but stimulated hyperactivation only weakly, in a dose-independent manner (concentration range 0.1–20 μM). At pH 8.5 (buffered with TAPS), 'control' levels of hyperactivation were increased fivefold (from 2.5 ± 0.66 to $12.29\pm1.82\%$), $n=21$, $P=2\times10^{-6}$) but when progesterone was applied the effect on hyperactivation was unchanged and sperm velocity decreased. At both pH 7.4 and 8.5 the effect of progesterone was inversely proportional to the spontaneous level of hyperactivation. These data confirm that activation of Catsper by progesterone only weakly enhances hyperactivation and that alkalinisation does not interact synergistically with progesterone to enhance hyperactivation of human sperm. The effect of pH 8.5 on hyperactivation may involve factors other than CatSper activation.

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O023

Notch signaling in the bovine oviduct and spermatozoa: a regulator of the spermatozoa-oviduct interaction?

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Gamete final maturation, fertilization, and embryo cleavage occur in the oviduct. It is known that oviductal luminal fluid composition differentially changes in the presence of oocytes, spermatozoa or embryos, prompting for a molecular cross-talk between the gametes/embryo and the oviductal epithelium. However, our understanding of the regulatory molecular mechanisms of gamete/embryo-maternal cross-talk is still fragmentary and controversial. The deciphering of these mechanisms could have a major impact on the optimization of assisted reproductive techniques and reproductive control strategies. The Notch cell signaling pathway is known to regulate cell communication in several embryonic and adult tissues. Our research goal is to evaluate the role of Notch signaling in gamete-maternal cross-talk in the bovine model. The expression of Notch components (receptors: Notch1–4; ligands: Dll1, 3–4, Jagged1 and 2; effectors: Hes1 and 2) was analyzed in the oviductal isthmus, ampulla, and infundibulum epithelia of prepubertal ($n=3$) and cyclic (estrous: $n=3$ and metaestrous phase: $n=3$) heifers by immunohistochemistry. Gene transcription/expression of Notch components was analyzed in ejaculated frozen-thawed spermatozoa by RT-PCR (Notch1–4, Dll1, Dll4; Jagged1 and 2) and immunofluorescence (Notch3, Jagged1 and 2). Notch1–3, Dll4 and Hes1 and 2 proteins are expressed in the epithelium of all oviduct segments, whereas Dll3 and Jagged2 are not expressed. The expression of Notch4 and Jagged1 is affected by estrous cycle stage and oviductal segment. Gene transcription and expression of Notch3 and Jagged2 was detected in spermatozoa, whereas Jagged1 is only found at the protein level. This temporal and spatial expression pattern of Notch components prompts for a regulatory role of Notch signaling in the oviductal epithelium and spermatozoa-oviductal cross-talk.

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O024

The effects of alcohol-induced oxidative stress on sperm DNA and fertility outcomes

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Background

A large population of apparently normal males have problem impregnating their partners even when their fertility status by physical examination and endocrine laboratory test is considered normal.

Aim

To investigate the effects of alcohol on sperm DNA and fertility outcomes.

Materials and methods

The experiment involved four groups of 20 adult male Sprague–Dawley rats which were randomly divided into groups of five rats each. The experimental animals were administered 30% v/v of ethanol at a concentration of 2 g/kg body weight while the control animals received distilled water. The experiment lasted for 4 and 8 weeks. At the end of each duration, the animals were introduced to female Sprague–Dawley rats on the pro-estrous day of their cycle. After a period of over 48 h the male animals were withdrawn, the testis and cauda epididymis harvested for oxidative stress levels and semen analysis respectively. Sperm DNA fragmentations were also measured.

Results

There was a significant decrease in sperm count, sperm motility, and the number of foetuses sired by the animals that received alcohol compared to controls. There was also a significant increase in malondialdehyde (MDA) levels, sperm DNA fragmentation of animals that received alcohol compared to controls.

Conclusions
Alcohol-induced oxidative stress increases sperm DNA fragmentation altering the ability of spermatozoa to fertilize oocytes and the sperm capacity to trigger the fertilized oocyte development for implantation.

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Oral Communications 4: Female reproduction

O025

Alteration of mechanisms underlying pituitary microvasculature remodelling and reduction in prolactin secretion precede full activation of the gonadotrophic axis in anoestrous ewes receiving exogenous melatonin

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In photoperiodic species, the pattern of pineal melatonin secretion translates the effects of day length on the annual reproductive cycle. Continuous delivery of exogenous melatonin to sheep during the non-breeding season is known to activate the gonadotrophic axis, and to suppress prolactin secretion through a mechanism involving the pars tuberalis (PT) of the pituitary gland. Recent studies have revealed that vascular endothelial growth factor (VEGF), a major regulator of angiogenesis and vascular permeability, is present in PT cells expressing MT1 melatonin receptors. In the current study, we investigated whether timely administered melatonin to ewes in the non-breeding season can induce differential expression of pro-angiogenic ($VEGF_{165}$) and anti-angiogenic ($VEGF_{165b}$) isoforms of VEGF in the pituitary and affect the prolactin and gonadotrophic axes. Melatonin was injected twice daily for 18 days during the spring; the injections (1.5 mg/dose, i.m.) were given in the afternoon (1530 h) and early morning (0500 h) to extend the nocturnal endogenous rise. Control animals received vehicle ($n=4$/group). Blood samples were collected every 3 h for 24 h on day 4 to determine prolactin concentrations, and every 10 min for 8 h on day 15 to determine concentrations of LH and FSH. The effects of treatment on pituitary VEGF isoform expression were investigated by immunohistochemistry. Melatonin significantly suppressed the nocturnal prolactin rise ($P<0.05$). The frequency of LH pulses was 1.5 ± 0.29 and 0.75 ± 0.48 pulses/8 h for melatonin and control ewes, respectively, whereas mean FSH concentrations for the same groups were 0.7 ± 0.16 and 0.58 ± 0.06 ng/ml; these differences did not reach statistical significance ($P>0.05$). However, $VEGF_{165b}$ expression was significantly increased in the pituitary of melatonin-treated animals ($P<0.05$). The results show that melatonin targets the mechanisms underlying pituitary micro-vascular remodelling and the prolactin axis before fully activating the gonadotrophic axis when timely administered to anoestrus ewes.

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O026

Neuroendocrine control of ovulation in primates: a role for two distinct populations of GnRH neurons?

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GnRH neurons represent the primary neuroendocrine link between the brain and the rest of the reproductive system, and traditionally it has been assumed that a single population of GnRH neurons controls both pusatile LH release as well as the preovulatory LH surge. This view has profoundly influenced our strategies for contraception and for the treatment of infertility in women. Recent data from our laboratory, however, questions the validity of this fundamental assumption. Using the female rhesus monkey as a translational animal model, we found that: i) primates express two distinct molecular forms of GnRH, both of which are highly effective at stimulating LH release; ii) GnRH-I and GnRH-II, are encoded on different chromosomes, and the neurons that secrete them have completely distinct locations in the hypothalamus; and iii) GnRH-I neurons respond to oestrogen exclusively in a negative manner, while GnRH-II neurons respond to oestrogen exclusively in a positive manner. Taken together, these data suggest that different aspects of reproductive function in primates are orchestrated by two distinct populations of GnRH neurons, with GnRH-II neurons playing the primary role in mediating the oestrogen-induced preovulatory LH surge. Moreover, these findings suggest that it may be possible to selectively silence this subpopulation of neurons in humans, using pharmacological agents – thereby blocking ovulation while leaving the rest of the reproductive axis relatively unperturbed.

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O027

Investigation into the role of endometrial heparinase, hypoxia-inducible factor 1A, secreted phosphoprotein 1, uteroferrin, and vascular endothelial growth factor A in foetal growth in pigs

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Low birth weight observed in the 'runt' piglet of the litter compared to its siblings has severe consequences for neonatal and adult development that cannot be remedied post-natally. This study compared the expression of a number of candidates hypothesised to be involved in angiogenesis and placental attachment in porcine endometrial samples.
Endometrial tissues supplying the smallest and normal-sized foetuses were collected from large white landrace gilts at gestational day (GD) 30 ($n=4$) and 60 ($n=4$). QPCR for secreted phosphoprotein 1 (*SPP1*), endometrial heparinase (*HPSE*), vascular endothelial growth factor A (*VEGFA*), uteroferrin (*ACP5*), and hypoxia-inducible factor 1A (*HIF1A*) was carried out and the relative expression of each candidate analysed. SPP1 protein localisation was quantified in paraffin embedded samples using immunofluorescence.
Relative expression of *ACP5* and *SPP1* was increased and *HPSE* was decreased at GD60 (3.26 ± 0.57; 2.52 ± 0.32; and 0.92 ± 0.25, respectively; mean $\pm$ S.E.M.) compared to GD30 (0.29 ± 0.08; 0.27 ± 0.07; and 3.20 ± 0.74; FPr ≤0.002). No size related differences in the expression of any of the genes was detected. Immunofluorescence revealed SPP1 protein is highly expressed in the glandular epithelium at GD60 ($9.91\%\pm1.08$), with little expression detected at GD30 ($0.15\%\pm0.07$; FPr <0.001). Endometrial samples supplying small foetuses had decreased SPP1 expression per glandular epithelium compared to those supplying normal foetuses. These findings suggest that temporal changes in endometrial expression of these candidates during gestation occurs, but is not altered in endometrial tissue supplying smaller foetuses. To fully understand the mechanisms governing growth retardation, both the endometrial and placental contribution must be thoroughly investigated.
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O028

Regulation of the prostaglandin synthase COX2 by epidermal growth factor requires steroid receptor coactivator interacting protein in human myometrium

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Human parturition at term and preterm involves inflammatory and non-inflammatory pathways and includes activation of the intrauterine prostaglandin (PG) cascade, resulting in synchronised membrane rupture, cervical dilation, and myometrial contractility. A key mediator of uterine PG production is the highly inducible enzyme cyclooxygenase 2 (COX2 or PTGS2). We have identified steroid receptor coactivator interacting protein (SIP or KANK2) to be highly expressed in human myometrium and fetal membranes, although its role in the uterus has yet to be investigated. siRNA-mediated knockdown of SIP in primary myometrial cells significantly reduced epidermal growth factor (EGF)-stimulated expression of COX2 mRNA and protein, while it was unable to decrease phorbol ester (PMA)-stimulated induction of COX2. EGF stimulation resulted in rapid and transient phosphorylation of SIP, which was blocked by pharmacological inhibition of the MEK/ERK signalling pathway with PD-184352. Moreover inhibition of ERK signalling decreased EGF-stimulated COX2 expression, suggesting that SIP phosphorylation plays an important role in its ability to regulate transcription of the *COX2* gene. The data support a role for SIP in the complex mechanism of human parturition.

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O029

Maternal leptin levels: association with pregnancy outcome

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Leptin is implicated to be altered in pathological pregnancies. We followed up 312 singleton mothers from 12 to 14 weeks gestation (booking) until delivery. Majority had a normal pregnancy ($n=253$). Others developed complications (gestational diabetes mellitus (GDM): $n=13$, pregnancy induced hypertension/pre eclampsia (PIH/PE): $n=27$, intrauterine growth retardation (IUGR): $n=17$, and other: $n=2$). Plasma leptin at booking visit and in the third trimester (referred to as term) were measured by ELISA. Leptin levels (booking visit and term) significantly differed between groups (geometric mean (95% CI) booking: 30.52 (25.69–36.26), 31.37 (29.16–33.74), 20.37 (29.16–33.74), and 13.86 (11.81–16.72) and term: 57.97 (51.11–65.75), 75.02 (69.41–81.08), 43.03 (41.50–44.61), and 26.36 (23.57–29.47) ng/ml in GDM, PIH/PE, normal pregnancy and IUGR respectively; Kruskal–Wallis ANOVA: $P<0.0001$). GDM and PIH/PE had higher leptin levels (Dunn's multiple comparison: $P<0.05$) than normal pregnancy and IUGR. Leptin levels were lower in IUGR than in normal pregnancy ($P<0.05$). On normalization to BMI, leptin levels were no longer significantly different between normal pregnancy and GDM (booking and term) or IUGR (booking). Maternal weight gain and birth weight were also significantly different between groups as expected (one-way ANOVA: $P<0.0001$) with former being higher in GDM and PIH/PE, and lower in IUGR. Birth weights were higher in GDM and lower in PIH/PE and IUGR. Higher leptin levels seen in early and late pregnancy in PIH/PE, and lower leptin levels seen in late pregnancy in IUGR even when accounted for BMI, suggest that maternal leptin levels are modulated by different factors in IUGR and PIH/PE despite both leading to low birth weight.

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Poster Presentations

P001

Sperm preparation technique affects functional motility in human sperm

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Human sperm use different behaviours for different aspects of sperm transport and fertilisation. We have investigated the effects of preparation method (density gradient method and direct swim-up) on sperm behaviour and manipulated Ca^{2+} signalling (the primary regulator of sperm motility) to induce different behaviours and assessed their effects on penetration of human sperm through viscous medium (methylcellulose).

Cells prepared by swim-up performed significantly better in penetrating methylcellulose than cells from the same sample prepared by density gradient centrifugation. CASA analysis showed that that swim-up cells had higher curvilinear and straight line velocities. In $[Ca^{2+}]_i$ imaging experiments cells prepared by density gradient included a higher proportion of dead sperm (assessed by retention of fluo4) and fewer cells that gave a $[Ca^{2+}]_i$ response to progesterone (fluo4 fluorescence) compared to cells prepared from the same sample using swim-up ($P<0.01$).

Treatment of cells prepared by density gradient with PGE_1 (to stimulate CatSper channels) increased curvilinear velocity ($P<0.01$; $n=9$) and enhanced performance in the viscous medium penetration assay ($P<0.0002$; $n=10$). Treatment with 4-aminopyridine, potently increased hyperactivation (assessed by CASA) and inhibited penetration of viscous medium in both density gradient and swim-up cells ($n=10$; $P<0.001$).

We conclude that i) cells prepared by density gradient centrifugation perform less well in penetrating viscous medium and are also less responsive to activation of CatSper by progesterone as measured by $[Ca^{2+}]_i$ and ii) penetration through viscous medium is inhibited by hyperactivation and enhanced by activation of CatSper (probably increased swimming velocity).

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P002

Associations between circulating non-esterified fatty acids and uterine function in *postpartum* dairy cows

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Evidence suggests that excessive non-esterified fatty acid (NEFA) production during *postpartum* negative energy balance (NEB) increases the risk of uterine infection. The aims of our study were to investigate associations between circulating NEFAs and uterine gene expression in *postpartum* dairy cows with NEB using microarray and bioinformatics techniques. Mild NEB ($n=6$) and severe NEB ($n=6$) in 12 cows were produced using different milking and feeding protocols. The cows were slaughtered at 14 ± 0.4 days *postpartum*. Circulating NEFA concentrations before slaughter were quantified. RNA extracted from uterine endometrium was analysed using Affymetrix 24K GeneChip Bovine Genome Arrays. Pearson correlation with Benjamini & Hochberg adjustment established the association between NEFA concentrations and normalized gene expression values. Ingenuity pathway analysis (IPA) was used to identify the biological processes, pathways, and networks. Circulating NEFA levels were significantly higher in SNEB than MNEB cows (1.4 ± 0.1 mmol/l vs 0.3 ± 0.1 mmol/l). Many uterine genes (1182) were significantly correlated with NEFA levels, of which about 80% were differentially expressed between SNEB and MNEB groups. The genes (806) with very large effect size ($|r|>0.6$) were selected for further analysis. Among them 253 were associated with inflammation, 178 with metabolism and 43 with reproduction. Most of the immune genes (196/253, 77%) were positively correlated with NEFAs, in which some genes involving in acute inflammation and infection, such as *SAA3*, *CXCL6*, *CFB*, and *IL8,* etc. had r values >0.8. IPA illustrated various aspects of immune/inflammatory processes, pathways and networks were on the top lists. Our results suggest that increased NEFA production in *postpartum* dairy cows interrupts uterine immune/inflammatory processes and predisposes the animals to uterine infection.

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P003

Characterization of migratory and gonadal porcine PGCs

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Primordial germ cells undergo an orchestrated developmental program prior to establishing the mature gametes. Most of our knowledge on PGC development stems from studies in mice, however recent studies in humans revealed important differences in the mechanisms of PGC development. Since pigs share many embryological features with non-rodent species, like humans, and human PGC can only be studied from early gonadal stages, we have used pig embryos to study the developmental program of PGC from their inception until they reach the gonad. Embryos collected between days 16 and 70 were sectioned and stained for a panel of markers used to identify PGCs as well as to establish their epigenetic features. Early migratory PGC were identified by expression of *Blimp1*, *AP2g*, *Sox17*, *Nanog*, and *Oct-4*, and absence of *Sox2*. These cells show reduced levels of DNA methylation (5-mC), which was consistent with low levels of Dnmt3a and UHRF, and increased levels of 5-hmC and tet-1. In gonadal PGCs, 5-mC and 5-hmC are both very low/absent in male and female PGC. H3K9me2 is absent/low in migratory PGCs and increases in gonadal PGC, although it remains lower than in somatic cells. H3K27me3 is detected in migratory PGCs and decreases in gonadal PGCs compared to somatic cells. Histone H2A.Z is detected in migratory PGCs, and the signal increases in late gonadal stages. This systematic characterization of porcine PGCs identified important differences in the program of germ cell development compared to mice, and similar patterns of gene expression and epigenetic marks to those reported for humans.

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P004

Effect of macrophages on steroidogenesis and cell migration in bovine ovaries

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Introduction

Cyclic ovarian function involves continual tissue remodelling. During ovulation macrophages invade the ovary and secret pro-inflammatory cytokines such us TNF- and IL6 that have local actions on ovarian cells. Here we investigated i) the effect of macrophages on secretion of oestradiol by granulosa cells (TC) and androstenedione by theca cells (TC) and ii) the effect of macrophages on TC and stroma cell (SC) migration was also assessed using an *in vitro* 'wound healing' assay.

Methods

Bovine monocyte-derived macrophages were prepared from citrated blood. GC and TC were isolated from 4 to 6 mm follicles and cultured (serum-free) for 4 days with/without macrophages in the presence/absence of LH (TC) or FSH (GC). Media were assayed for steroids and RNA extracted for gene expression analysis using RT-qPCR (normalized to β-actin). For wound-healing ('scratch') assays TC and cortical SC were cultured (10% serum) with/without macrophages for 2 days. A 'scratch' was made in the near confluent monolayer and cell migration (% wound closure) assessed over 24 h.

Results and discussion

Macrophages suppressed FSH-induced estradiol secretion by GC ($P<0.0001$) and LH-induced androstendione secretion by TC ($P<0.001$). The inhibitory action of macrophages on GC was accompanied by down-regulation ($P<0.001$) of CYP19A1 (tenfold).and up-regulation of TNC and SLPI expression. In TC, macrophages reduced expression of several transcripts including INSL3 ($\sim$15-fold), CYP17A1 (approximately sixfold), LHR (fivefold), and HSD3B (fivefold). Co-culture of both TC and SC with macrophages accelerated wound healing ($P<0.001$) while dexamethasone (10^{-8}–10^{-6} M) retarded wound healing. Interestingly, exogenous TNC also accelerated wound healing in both SC and TC monolayers ($P<0.0001$) suggesting an involvement in the response to macrophages.

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P005

A deep RNA sequencing study of mammalian sperm RNA: identifying common cross-species expression motifs indicating functionality

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Despite being transcriptionally silent,[1] mature spermatozoa contain small amounts of RNA comprising various classes and although, *de novo* translation of spermatozoal RNA has been demonstrated,[2] the role of these RNAs is currently unclear. However, looking across species boundaries and comparing the RNA profiles of sperm from a number of mammals may give insights into their function in the process of reproduction, including potentially post-fertilisation requirements.[3]

High resolution RNA profiling by next-generation RNA sequencing (NGS) can provide information on the historical record of gene activity during spermatogenesis and can offer insights into functional aspects of sperm RNA in both spermatogenesis and potentially in embryogenesis.[4,5]

This study aims to compare RNAs from bovine, ovine, porcine, and human spermatozoa alongside corresponding samples of testis tissue. Following NGS, comparisons are being made by bioinformatic analysis aimed at the identification of shared gene expression networks that are indicative of conserved functionality across the tested species and sperm RNAs that are unique to or are highly elevated in sperm compared with testis. The focus initially, relies on analysis of mRNAs and long non-coding RNAs and their roles in both spermatogenesis and fertilisation.

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P006

Endometrial level of lysophosphatidic acid (LPA) and expression of LPA receptors mRNA in endometrosis in the mare

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Lysophosphatidic acid (LPA) is a bioactive lipid mediator that exerts a wide range of biological actions. LPA acting specifically through LPA receptor 1 (LPAR1) seems to be essential for the development of fibrosis in several organs. We determined whether the LPA–LPARs signalling system correlates to equine endometrial fibrosis (endometrosis). A total of 24 uteri from diestrus ($n=6$ for each category: I, II A, II B, and III according to Kenney and Doig classification) and 20 uteri from oestrus ($n=5$ for each four endometrosis category) were used for this experiment. The level of LPA and expression of four types of LPAR (1–4) were investigated in the endometrial tissue in the course of endometrosis. Additionally, the effect of LPA (10^{-9} M) on secretion of connective tissue growth factor (CTGF) and prostaglandin E_2 (PGE_2) from endometrial tissue at different stages of endometrosis was determined. The *LPAR1–4* mRNA transcription was not correlated with endometrosis development. In diestrus, LPA up-regulated CTGF secretion in category III compared to the control ($P<0.05$). In diestrus, LPA up-regulated endometrial PGE_2 secretion in all categories, while in oestrus it up-regulated PGE_2 secretion only in category I endometrium ($P<0.05$). There is no strong positive linking between LPA level and LPAR1–4 expression in endometrosis, but LPA up-regulated endometrial CTGF secretion in severe stage of fibrosis in diestrus. Moreover, the endometrial LPA level and LPA-stimulated PGE_2 secretion was impaired in estrus in the course of endometrosis what may disturb homeostasis essential in physiological processes occurring in endometrium. Supported by grant MAESTRO of National Research Center (2011/02/A/NZ5/00338).

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P007

Effect of hyaluronan on some molecular markers of implantation in sheep endometrial cells *in vitro*

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Previous studies indicate a pattern of expression for hyaluronan (HA) synthases in the reproduction system regulated by steroid hormones which produce different HA sizes with diverse physiological functions. The objective of the present study was to determine the effect of low molecular weight (LMW) and high molecular weight (HMW) HA on molecular markers of embryo implantation using *in vitro* model of endometrial cell culture system. Sheep endometrial cells at confluence were treated with 1 mg/ml LMWHA or 1 mg/ml HMWHA or 4-methylumbelliferone (4-MU, 1 mM) or the basic culture media as the negative control for 24 h. mRNA expressions of adhesive molecule cluster domain 44 (*CD44*) and anti-adhesive gylocprotein mucin 1 (*MUC1*), the level of phosphorylation of MAPK1/3 proteins and the amount of prostaglandins ($PGF_{2\alpha}$ and PGE_2) produced into the media were determined. LMW and HMW HA supplementation significantly increased *CD44* expression ($P<0.05$) in the endometrial cells compared to the control, whereas 4-MU treatment had no effect. In contrast to HMWHA which increased *MUC1* mRNA expression ($P<0.01$), LMWHA reduced ($P<0.05$) it as compared to the control. LMWHA increased total and phosphorylated levels of MAPK1 and MAPK3 levels. 4-MU treatment reduced PGE_2 production ($P<0.05$) by the endometrial cells, whereas HA supplementation did not influence PGs production. Similar molecular changes were reported at the feto-maternal interface *in vivo*. Modulation of molecular markers of embryo implantation in a pattern that enhances implantation as demonstrated in this study may be one of the possible mechanisms through which HA facilitates blastocyst implantation.

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P008

Expression of porcine CXADR and its role in blastocyst formation

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Coxsackie virus and adenovirus receptor, CXADR (CAR) is a member of the tight junction protein (TJP also known as JAM) family of adhesion receptor and located on a cytoplasmic membrane surface of intercellular tight junctions. CXADR are reported to be expressed during preimplantation in human embryos, but its function in early embryo development was not investigated. In the present study we determine temporal and spatial expression patterns of CXADR in porcine embryos and investigated the biological function in blastocyst formation. Porcine CXADR was detected in all stages of preimplantation, and highly up-regulated from eight-cell stage onward, particularly CXADR protein was translocalised into intercellular boundary at morula and blastocyst. To deplete both maternally and zygotically expressed CXADR, an RNAi approach was employed. Microinjection of CXADR dsRNA resulted in a ~90% reduction of mRNA and complete loss of protein. In CXADR knockdown (KD) embryos, blastocyst development was significantly lower (11.26% vs 38.32%) and most embryos were arrested prior to morula. We also examined genes required for and involved in tight junction biogenesis using qRT-PCR. Expression of tight junction associated genes such as ZO-1, and OCLN were decreased but TFAP2C, OCT4, and PARD6B were not changed. In conclusion, our results suggested that CXADR plays a critical role in blastocyst formation in terms of establishment of tight junction complex.

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P009

A model of tissue-engineered (3d) decidua to study the effects of environmental pollutants on endometrial physiology

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The increasing exposure to environmental chemicals is a burden for human reproduction, and can alter endometrial functions and maternal–embryo interactions. The available *in vitro* endometrial models often fail to represent the complexity of cellular environment, cells shape and organization, and could give misleading results on the effects of environmental chemicals. Here, a tissue-engineered (3D) decidua was used as a toxicological model. Human stromal cells were isolated from healthy endometria ($n=3$). Cells were vimentin positive and cytokeratin-7 negative. To build the 3D decidua, endometrial stromal cells were embedded in a fibrin-agarose matrix and decidualized *in vitro* with steroid hormones (17β-estradiol and progesterone). The 3D decidua was compared to bi-dimensional (2D) cultures of *in vitro* decidualized endometrial stromal cells. The secretion of IGFBP-1 was monitored up to 12 days, during the decidualization. The two models were then exposed to an environmental chemical, Bisphenol A (BPA, 1 nM) or to the vehicle (control =0.1% ethanol). The secretion of the cytokine macrophage migration inhibitory factor (MIF) was monitored as a marker of pro-inflammatory response. In a 3D environment, cells were more responsive to hormonal stimuli, as the decidualization was faster compared to the 2D cultures. MIF secretion increased upon exposure to BPA in both models, the effect being more significant in the 3D decidua ($P<0.05$). This study could open to new scenarios in toxicological studies, as the use of more complex *in vitro* models could help to better understand the effects of environmental pollutants on human health.

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P010

Characterisation of the dog as a sentinel species for human exposure to endocrine disrupting chemicals

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Introduction
A temporal decline in canine male fertility parallels that reported in the human. Our hypothesis is that this is associated with exposure to environmental chemicals (ECs). We investigated i) a relationship between canine testicular chemical profiles and Sertoli cell numbers and ii) the effects of testicular concentrations of chemicals on sperm DNA fragmentation, motility and vitality.
Methods
Canine adult testes (routine castrations) from the West Midlands (WM: $n=26$), East Midlands (EM two regions: $n=5$, $n=7$) and SE England (SE: $n=15$) were analysed for seven PCB congeners, seven PBDE congeners and DEHP. Sertoli cell numbers, identified by vimentin immunohistochemistry, were counted and adjusted for tubular area ($n=5$/region). Canine sperm samples ($n=10$), were cultured with PCB153 and DEHP (0, 2×, 10×, 100× mean testicular levels). Vitality, motility and sperm DNA fragmentation were analysed at 0, 120 and 240 min.
Results
Mean testicular levels for PCB 153 and DEHP were 0.063 ± 0.010 μg/kg ($n=35$) and 0.182 ± 0.044 μg/g ($n=26$) respectively. Higher levels of PCB and PBDE were found in WM testes ($n=12$) (?PCB: $P<0.05$, ?PBDE: $P<0.01$) than in other regions (EM: $n=9$, SE: $n=14$). WM testes also had the lowest Sertoli cell numbers ($P<0.05$). MTL of PCB153 or DEHP individually increased sperm DNA fragmentation ($P=0.055$) and reduced sperm vitality and motility ($P<0.001$).
Conclusion
Regional differences in testicular ECs, their association with reduced Sertoli cell numbers and their ability to directly affect sperm quality suggests that declining canine fertility has an environmental aetiology.

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P011

Assessment of major reproductive problems and reproductive status of crossbred (Holstein Friesian X Zebu) dairy cattle in and around Mekelle, Tigray, Ethiopia

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Ethiopia maintains an extensive livestock population, however reproductive performance of cattle and their breeding management are unsatisfactory. Currently, the sole diagnostic tool in the country is rectal palpation, which is inaccurate for early pregnancy, and causes embryonic and fetal loss. This study assessed major reproductive problems using questionnaire survey, and trialled simple, cost-effective alternative monitoring approaches using on-farm diagnostic tools to determine milk and serum progesterone and evaluate reproductive status. 177 dairy farms (range 1–115 cattle per farm) were included in the questionnaire survey. Of these, 47 participated in the on-farm diagnostic trial, and reproductive status of 319 crossbred (Holstein Friesian X Zebu) dairy cattle was assessed. Progesterone was measured by qualitative (Target P4) and Dip-stick (P4 Rapid) ELISA tests. Questionnaires indicated anestrus (37.8%), repeat-breeder (21.0%), dystocia (11.6%), retained fetal membranes (11.5%), endometritis (6.6%) and abortion (6.4%) as the major reproductive problems in the area. Together, progesterone ELISA and rectal palpation indicated in-heat 10 (3.1%), anestrus 77 (24.2%), repeat-breeder (follicular cyst) 9 (2.8%), normally cycling 69 (21.6%) and pregnant 154 (48.3%). Of 118 animals reported by farmers (questionnaire) as anestrus, only 72 (61.0%) had true anestrus ($P<0.05$) while the remaining 1 (0.9%), 39 (33.0%) and 6 (5.1) were repeat-breeder, normally cycling and pregnant, respectively. Of 171 animals reported by farmers as pregnant, the progesterone test showed 4 (2.3%) anestrus, 1 (0.6%) repeat breeder, 19 (11.1%) normally cycling and 147 (86%) pregnant. Target P4 ELISA and Dipstick are equally effective methods to assess reproductive status. Application of these tests by veterinarians, artificial insemination (AI) technicians and low-income smallholder farmers offers practical means to monitor reproductive status in cattle and improve breeding management to the benefit of the sector.

Keywords: Breeding, Cattle, Dipstick, Mekelle, Reproductive problems, Target P4 ELISA
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P012

Expression and possible role of receptor-interacting protein kinase (RIPK) 1 and 3 in the bovine CL

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Programmed necrosis or necroptosis is an alternative form of cell death that is regulated by a caspase-independent pathway. The aim of the study was to determine if necroptosis participate in bovine CL luteolysis. In Experiment 1. the *RIPK1* and *RIPK3* mRNA transcription was determined in bovine i) CLs from early, developing, mid, late and regressed-stage ($n=4$ for each stage), ii) CLs after colpotomy collected within 0-, 2-, 4- and 12-h (each $n=4$) after intramuscular prostaglandin F2α (PGF) injection on Days 10–12 of oestrous cycle, iii) luteal steroidogenic (LSC) and endothelial (LEC) cells from mid luteal phase *in vitro*. In Experiment 2. Mid-CL-derived LSC were

cultured with PGF (1.0 μM), TNF (2.3 nM) and/or interferon γ (IFNG: 2.5 nM) for 12, 24 and 48 h. Moreover, LSC were treated by TNF?IFNG with or without inhibitor for RIPKs, necrostatin-1 (Nec-1), in dose-dependent manner for 24 h. After culture, cell viability and mRNA transcription of *CASP3*, *CASP8* were determined. *RIPK1* and *RIPK3* were up-regulated: i) in late and regressed CL compared to early and developing CL ($P<0.05$) and ii) in CL collected within 4- and 12-h after PGF injection ($P<0.05$). TNF in combination with IFNG increased *RIPK1*, but not *RIPK3*, expression after 24 h in LSC ($P<0.05$). Although high concentration of Nec-1 prevented LSC from TNF?IFNG-induced cell death ($P<0.05$), it didn't affect *CASP3* and *CASP8* expressions. These findings suggest that RIPKs-dependent cell death can be one of potent mechanism of bovine CL regression.

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P013

Whether equine Corpus Luteum is a site for LPA synthesis and/or a target for LPA action?

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Lysophosphatidic acid (LPA) is a simple phospholipid and exerts various biological functions effecting on reproductive processes in rats, pigs, ewes and cow. LPA acts through three subtypes of Edg family G protein-coupled receptors (LPA1-3) and 'non-Edg family' LPA receptors (LPA4-6). The aim of the study was to determine LPA: i) concentrations and its receptors mRNA transcription, and ii) effect on P_4 secretion from equine CL during the oestrous cycle and early pregnancy. CLs were obtained in early-, mid-, and late luteal stages of the cycle and at Day 28 of pregnancy ($n=6$, each stage). In experiment 1. the concentration of LPA extracted from CL tissue was measured using ELISA. Additionally, the expression of LPA receptors (1–4) was determined by qPCR. In experiment 2. the CL explants were cultured for 24 h with LPA (10^{-9}M) and concentration of P_4 was determined using EIA. The concentration of LPA was up-regulated in the mid CL, and down-regulated in the early and pregnant CL ($P<0.05$). The mRNAs transcription of *LPAR1-4* were up-regulated in the late luteal phase compared to early, mid luteal stage of the cycle and pregnancy ($P<0.05$). Moreover, LPA stimulated P4 secretion from early and mid CL explants compared to other phases ($P<0.05$). We showed for the first time the presence of LPA and its receptor expression in equine CL. The results indicate that concentration of LPA and *LPAR1-4* mRNA transcription in equine CL is estrous cycle-dependent and LPA affect P_4 secretion during development of CL.

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P014

The effect of *E.coli* lipopolysaccharide (LPS) on bovine luteal endothelial cell network formation and steroidogenesis *in vitro*

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In dairy cows, *post-partum* uterine inflammation caused by Gram negative bacteria (e.g. *E. coli*) suppresses follicular function and reduces fertility. LPS is an endotoxin that is present on outer membrane of Gram-negative bacteria. Furthermore, LPS is detected in follicular fluid (0.04–0.88 μg/ml) of cows with endometritis. This study tested the hypothesis that LPS would decrease the formation of luteal endothelial cell (EC) network and progesterone production *in vitro*. Luteal cells (EC, steroidogenic luteal cells, and pericytes) were enzymatically dispersed from abattoir-derived bovine corpora lutea (early or mid-luteal phase) and incubated at 39 °C in 5% CO_2. On day 1 and thereafter, cells were treated with LPS (0, 0.01, 0.1, 1 and 10 μg/ml) under basal and angiogenic-stimulated conditions (FGF2 plus VEGFA; both 1 ng/ml). Spent media was analysed for progesterone by ELISA and replaced every 2 days. On day 9, ECs were immunostained for von Willebrand factor and EC networks quantified by image analysis. In control wells, EC formed highly organised tubule-like networks, which was dramatically increased under angiogenic stimulated conditions. LPS dose-dependently decreased the total area of EC network, the number of EC clusters and branch points as well as the degree of branching per EC cluster ($P<0.001$), even detectably lower at 0.01 μg/ml ($P<0.05$). This pattern was observed under both basal and angiogenic-stimulated conditions. Surprisingly, *in vitro* progesterone production was unaffected by LPS but was increased under angiogenic stimulated conditions ($P<0.001$). In conclusion, LPS dramatically inhibited luteal endothelial cell network formation in a dose dependent manner *in vitro*.

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P015

The association between uterine disease and subsequent reproductive performance in commercial UK dairy herds

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Dairy cow fertility has declined over the past 50 years coinciding with increased milk production. Uterine health is an important factor with endometritis prevalent in high-yielding dairy cows. This study assessed the association between uterine disease on reproductive performance in 78 commercial UK dairy herds. Data from 59 118 lactations ($n=29$ 157 cows) was collected from 2000to 2009 and included presence of uterine disease, calving date, and insemination information. Linear mixed model analyses were performed to determine the association between cows experiencing uterine disease their conception rate, days to first insemination (DFS), calving to conception interval (CCI) and calving interval (CI). The proportion of animals culled was compared using χ^2-test. The mean incidence of endometritis was 12% per lactation. The DFS, CCI and CI were extended by 7 ($P<0.05$), 20 days ($P<0.001$) and 26 days ($P<0.001$), respectively in cows, which had experienced uterine disease in that lactation. The extension in the CCI was, in part, explained by a lower first service conception rate ($P<0.0001$) in cows that had *post-partum* uterine disease (24.3%) compared to controls (38.0%). Furthermore, there was an increase of 0.8 services per conception ($P<0.001$). The culling rate in cows that experienced uterine disease was greater (24.9%; $P<0.001$), compared with the control group (21.2%). This study has quantified the negative associations between cows experiencing uterine disease and subsequent reproductive performance in UK commercial dairy herds, with the biggest relationship appearing to occur on the ability of cows to re-conceive.

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P016

Membrane potential is crucial for one of the $[Ca^{2+}]_i$ oscillations profiles induced by progesterone in human spermatozoa

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Ca^{2+} signalling is critical for regulation of sperm motility. $[Ca^{2+}]_i$ oscillations, which may underlie observed 'switching' of sperm behaviors, occur in human spermatozoa stimulated with progesterone. Our work aimed to investigate the potential contribution of changes in membrane potential, leading to cyclical activation of voltage dependent Ca^{2+}-influx pathways, to $[Ca^{2+}]_i$ oscillations. Spermatozoa were incubated with fluo4-AM and clamping of membrane potential to E_K was performed using K+ ionophore valinomycin (VLN–1 μM) before or during progesterone (P4–3 μM) treatment. The cell population exhibiting $[Ca^{2+}]_i$ oscillations after P4 stimulation was 29.8 and 25.4% (4 and 7 h capacitation, respectively). Interestingly, two different oscillations patterns were observed: 'rapid' transients with amplitude similar to the initial progesterone response and slower transients with lower amplitude. Pre-treatment with VLN abolished 'rapid' $[Ca^{2+}]_i$ oscillations in 95% of cells, however, 'slow' transients were not sensitive to VLN. Moreover, VLN did not prevent nor alter the amplitude of the initial transient response induced by P4. Similar results were obtained when VLN was added after P4 stimulation. 98% of cell

population had $[Ca^{2+}]_i$ oscillations inhibited in the presence of VLN but 'slow' transients were resistant to membrane potential hyperpolarization. Both Ca^{2+} oscillations patterns were completely recovered when VLN was removed. Our results showed that membrane potential contributes to $[Ca^{2+}]_i$ oscillations generation but only to high amplitude transients, suggesting that CatSper may be in involved in this oscillation profile, potentially triggering CICR. Low amplitude transients may be generated by a second Ca^{2+} signalling pathway.
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P017

Differential proteomic profiles of porcine follicular fluid associated with a high fibre diet and later fertility

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In a previous study, following IVF, oocytes from gilts fed a high fibre diet for the first 19 days of their third oestrous cycle, produced blastocysts with more cells than oocytes from control-fed gilts. We hypothesise that FF protein composition is altered by the diet and that this confers the reproductive benefits.
The current study compared the protein composition of pooled Day 19 FF from 12 high fibre-fed pigs and 12 control-fed pigs in search of biomarkers for fertility or diet. Within each dietary group, the protein composition of pooled FF from pigs whose oocytes produced blastocysts was compared with FF from pigs whose oocytes did not produce blastocysts ($n=6$/group). The proteomic study was carried out in duplicate.
Abundant proteins were depleted from FF samples by Proteominer enrichment. Remaining proteins were labelled by di-methylation and detected by liquid chromatography tandem mass spectrometry. Differentially expressed proteins were submitted into ingenuity pathway analysis (IPA) and a process of biomarker candidate selection was carried out.
The study detected over 140 differentially expressed proteins between control and high fibre FF samples, indicating a nutritional influence on FF protein composition. Several of these proteins were also differentially expressed in the blastocyst versus no blastocyst analyses, suggesting that nutritionally altered FF protein composition may affect IVF outcome. IPA analysis revealed the association of differentially expressed proteins with several molecular pathways, upstream regulators and networks. Quantitative Western Blot will be carried out for confirmation of selected differentially expressed proteins.
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P018

An E4BP4 knockout model to assess the distribution of uterine Natural Killer cell subsets in mouse pregnancy

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Natural Killer cells have been linked to a number of disorders of pregnancy by both mouse studies and association studies in humans, with NK cell dysfunction leading to reduced spiral artery remodelling. However, little research has centred on the importance of the newly divided subsets of uterine Natural Killer cells, some of which appear to develop independently of E4BP4, in spiral artery remodelling, and subsequently fetal growth. Hence, we sought to analyse the dependence of different uterine subsets of Natural Killer cells on E4BP4 by use of a knockout model. Our data show that the $E4bp4^{-/-}$ mouse possesses uterine NK cells and may show an altered distribution of DBA? NK cells compared to C57BL/6. Further investigation is needed to account for decreased vascular remodelling and fetal growth in this knockout model.
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P019

PCR detection and phylogenetic analysis of bovine herpesvirus 4 field-isolates from dairy cows in Thailand

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Introduction
Postpartum dairy cows are susceptible to uterine infections that leading to poor fertility. During this period the uterine defence mechanisms were compromised by poor energy status and the preceding viral infections, e.g. bovine herpesvirus 4 (BoHV-4) resulting in uterine pathology initiated by microorganisms. BoHV-4 has been isolated from healthy cows and cows experienced with mastitis, metritis and endometritis worldwide.
Methods
Samples of bulk tank milk, blood and endometrium were collected from four commercial dairy herds located in the centre of Thailand. After viral DNA extraction, PCR was performed to detect BoHV-4 glycoprotein B (gB) DNA, and nested-PCR was performed for the detection of BoHV-4 thymidine kinase (TK) DNA. The retrieved amplicons were sequenced, aligned and compared with other available BoHV-4 DNA sequence data from GenBank.
Results and discussion
Amplicons of the gB and TK DNA were evidenced in bovine endometrial samples and blood, but not in bulk tank milk. To our knowledge, this is the first report of BoHV-4 isolated from dairy cows in Thailand. Phylogenetic analyses showed that the BoHV-4 TK DNA recovered from bovine endometrium in Thailand shared similarity with the Argentinean strains obtained from vaginal discharge of aborted cows and Brazilian strain obtained from central nervous system, while the BoHV-4 gB DNA recovered from blood located in a distinct branch compared with other sequences from Germany, Ireland, Belgium, Brazil and Turkey. An assessment of genomic survey of BoHV-4 and its correlation with uterine pathology may improve the strategies of reproductive management in the dairy industry.
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P020

The effect of oocyte-specific ablation of *N*- and *O*-glycans on the cumulus extracellular matrix

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Each egg, when ovulated from a follicle, is surrounded by cumulus cells. Prior to ovulation, these cumulus cells secrete cumulus extracellular matrix (cECM) molecules, resulting in cumulus expansion. Cumulus expansion has been linked to the developmental quality of the oocyte. Hyaluronan (HA), the major constituent of the cECM, is stabilised by molecules such as heavy chains (HCs), pentraxin 3 (PTX3) and tumour necrosis factor-stimulated gene 6 (TSG6) during expansion. All of these molecules, except HCs, are secreted by the cumulus cells and are dependent on oocyte-secreted factors, such as bone morphogenetic protein 15 (BMP15) and growth and differentiation factor 9 (GDF9), both of which signal via SMAD pathways. The Double Mutant mouse model (DM), with oocyte-specific deletion of *C1galt1* and *Mgat1*, has altered cumulus expansion without affecting fertilisation. We investigated whether the absence of oocyte-specific complex *N*- and *O*-glycans in DM affected levels of HA, HCs, PTX3, TSG6, pSMAD1/5/8 and pSMAD2 using immunohistochemistry in cumulus-oocyte complexes (COCs) at 48h post PMSG and 9 h later, post hCG stimulation. DM COCs did not differ in cumulus size or cell density at either 48 h post PMSG or 9 h post hCG stimulation compared to Controls. However, HA, HC and pSMAD1/5/8 levels were reduced in DM COCs compared to Controls. No significant correlations were found linking the matrix molecules with pSMADs, or with cumulus area. We propose that although oocyte-specific ablation of *C1galt1* and *Mgat1* reduces cECM levels of HA, HC and pSMAD1/5/8 expression, the levels are sufficient for cumulus expansion.
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P021

Inter-generational effect of maternal analgesic exposure on female ovaries of second generation of female rats

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Analgesics which work through altering the production and/or actions of prostaglandins (PGs) are widely used by pregnant women. In earlier research, the analgesics in maternal blood circulation can target cyclooxygenase-2 and PGE2 receptors in fetal germ cells (GC), affect the next generation. Both sexes of F1 rats exposed to analgesic *in utero* showed reduced germ cell (GC) number in gonads at the fetal stage, and reduced ovarian oocyte reserve in adult stage and/or in the development of the postnatal ovary and folliculogenesis/ovulation in female offspring. Similarly, reduced ovarian weight was observed in female F2 offspring of F1 parents that had been exposed to analgesics. This shows that maternal analgesic exposure does have an inter-generational effect on females in the next generation.

To investigate whether changes in ovarian weight was paralleled with impairment of reproductive system. Three main approaches were performed on the morphology analysis of ovaries at different stage and ovarian reserve. The results in this thesis demonstrated that F1 maternal analgesic exposure did have effect on structure and function of reproductive system of F2 offspring, in puberty stage, female offspring had reduced size of primordial follicle pool, while in adult stage, serum level changed in analgesic exposed F2 offspring. However, whether fertility ability of F2 offspring was impaired needs further studies to figure out the dynamic of oocyte and what leads to the changed profiles of follicle composition.

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P022

Role of sperm thiols? redox status in keeping rat sperm quiescent in cauda epididymis

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Background
The sperm require energy-intensive motility to reach the female gamete for delivering the male genome. This energy is conserved by keeping the sperm quiescent in cauda epididymis before ejaculation, but the molecular mechanisms controlling this unique process remain an enigma. Cauda sperm produce H_2O_2, and we have attempted to study the redox regulation of sperm motility.
Methods
Quiescent and motile sperm were collected from Sprague Dawley rat cauda epididymides and experimented *in vitro*. Care was taken to prevent motility initiation of quiescent sperm on isolation from epididymis.
Results
Free thiols on quiescent sperm increased by ~2-fold on motility initiation. Caudal sperm failed to initiate motility in presence of 0.1% sulfhydryl-alkylating agent N-ethylmaleimide (NEM), and when applied vaginally before mating 50 mg NEM prevented pregnancy in rabbits. While ~50% motile-sperm lost complete motility when placed in0.05% H_2O_2, 50% quiescent-sperm did not initiate any motility at 0.02% H_2O_2. Similarly, all motile sperm could be immobilized by at least 2.0% H_2O_2 while none of the quiescent sperm could initiate motility at 0.25% H_2O_2. However, caudal sperm could initiate good motility at pH (6.85) and viscosity (82 cP) of the caudal semen.
Conclusions
The redox potential of sperm thiols may play a crucial role in rat sperm quiescence and motility initiation. The pH and viscosity of caudal semen may not have a critical effect on sperm motility suppression. Quiescent sperm are more susceptible to these factors than motile sperm.

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P023

The effects of environmental chemicals on bovine luteal function

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Dairy herd fertility is in decline and poses a significant economic problem, with associated animal welfare concerns. One factor that may contribute to this decline is exposure to environmental chemicals (ECs) some of which have endocrine disrupting activity and have been linked to declining fertility in other species. We hypothesised that ECs are present in bovine ovarian tissue at levels able to perturb luteal development and function. The tissue content of DEHP and a panel of PCB congeners was determined in abattoir-derived bovine ovaries ($n=4$), following tissue extraction and gas chromatography linked to mass spectrometry (GC–MS). Both DEHP and several PCBs were detected in bovine ovarian tissue. DEHP and PCB153 were detected well within the analytical range (1.51 μg/g and 0.039 ng/g respectively), whilst mean concentrations of PCBs 52, 101, 118, 138, 153 and 180 were just above the detection limit of 0.02 ng/g. Due to the importance of angiogenesis in luteal growth and subsequent progesterone secretion, the dose-dependant effect of DEHP was further investigated utilising a bovine luteal angiogenesis culture system ($n=3$). DEHP did not alter the degree of luteal endothelial cell network formation or progesterone production over 9 days in culture. In conclusion, ECs are present in the bovine ovary and DEHP, at environmentally relevant concentrations, does not appear to influence luteal angiogenesis or steroidogenesis *in vitro*. Other individual and combined ovarian ECs are under investigation.
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P024

Ovarian transcriptome profile before, during and after the onset of premature ovarian failure in a mouse with oocyte-specific deletion of *Mgat1* and *Cgalt1* genes

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Premature ovarian failure (POF) affects ~1% of women over 40 and is idiopathic in 74–90% of cases. A transgenic mouse model of POF has been generated (known as double mutant; DM) resulting from oocyte-specific deletion of two glycosyltransferases. Glycoproteins from DM oocytes lack complex *O*- and *N*-glycans. DM females are fertile at 6-weeks, infertile by 9-weeks and exhibit POF by 12-weeks of age with follicle depletion, elevated gonadotropins and decreased sex steroids. To identify possible genetic pathways involved in the onset of POF, we analysed Control and DM ovary transcriptomes at 3-, 6- and 9-weeks of age. Ovaries were collected and pooled in triplicates ($n=3$ per group; three groups), total RNA extracted and whole-genome expression profile carried out using Illumina arrays. Data analysis was performed using R. A total number of 21 007 probes were assessed and differentially expressed genes detected at 9-weeks were analysed for functional enrichment using DAVID. Transcriptomic analysis of DM ovaries demonstrated a sharp change in gene expression compared with Controls from 3- to 9-weeks. In DM ovaries, only two genes were detected as differentially expressed at 3-weeks (*Snrpa*; down-regulated and Ctsk; up-regulated) and three more genes at 6-weeks (*Kntc1*, *Dppa5a* and *RIKENcDNA E330017A01*; all of them downregulated) which could represent candidate gene regulators for the onset of POF in the DM. A total of 1253 genes were differentially expressed at 9-weeks in DM ovaries. Up-regulated genes were preferentially enriched for cell communication and extracellular matrix, representing potential pathways affecting ovarian function and impairing fertility capacity.

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P025

***CG9879* in the *Drosophila* testis**

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Abstract
In *Drosophila,* the testis meiotic arrest complex (tMAC) regulates the expression of a vast number of genes involved in spermatogenesis and is essential for entry into male meiosis. Among its many targets are a few putative transcription factors that could further regulate spermatogenesis genes. *CG9879* is implicated as one such tMAC-induced transcription factor as it is testis-specific, tMAC-dependent, and homologous to the known transcription factors *TBP, TRF* and *TRF2*. In this project the expression pattern of *CG9879*was examined on a cellular level using whole mount *in-situ* hybridisation of testes and found to be restricted to mid-late spermatocytes. The homologues were also examined, and *TRF2* was found to be expressed in earlier spermatocytes. Furthermore, the sub-cellular localisation of CG9879 protein was examined by expression of a *GFP-CG9879* transgene. Contrary to expectation, GFP-CG9879 was not nuclear-enriched, casting doubt upon the putative role of CG9879 as a transcription factor.

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P026

Regulation of expression of oocyte quality control factor, ATRX, in bovine oocytes during maturation

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In humans, mutation of the ATRX gene leads to improper methylation of repetitive DNA sequences. By performing a meta-analysis on published microarray data across several model species we previously identified ATRX as a potential biomarker of oocyte quality. The aim of the present study was to determine the expression and regulation of ATRX at a protein level, in the bovine cumulus oocyte complex (COC).
ATRX protein was found to be expressed during oogenesis and to be dramatically downregulated during oocyte maturation. We have previously shown that inhibition of progesterone signalling during bovine *in vitro* oocyte maturation has a detremental affect on subsequent embryonic development. Here we show that this is characterized by to an increase in ATRX expression and a parallel increase in the expression of a known marker of apoptosis (active Caspase-3) during oocyte maturation, in both oocytes and cumulus cells.
Immunohistochemistry studies performed on bovine oocytes at different stages of meiotic maturation showed ATRX to be localized to the chromosomal area of GV

oocytes, but undetectable in MII oocytes. Inhibition of progesterone signaling during maturation resulted in the stabilization of ATRX in bovine oocytes, with ATRX remaining localized to the chromosomal area of mature MII oocytes.
In conclusion, ATRX protein expression and localization appears to be progesterone regulated and associated with oocyte quality. However, further work is needed to determine how the regulation of ATRX corresponds to specific epigenetic regulations and subsequent developmental competence.

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P027

Dynamics of hyaluronic acid (hyaluronan) binding proteins in relation to sperm capacitation and the acrosome reaction

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Objective
Considering the recent interest in the use of hyaluronan binding as a sperm selection marker for ICSI, it is becoming increasingly important to revisit and characterise the sperm hyaluronic acid binding proteins (HABPs) that are an integral part of the selection process. To this end, the HABPs in non-capacitated, capacitated and acrosome reacted spermatozoa were investigated.
Design
A laboratory based investigation using immune-cytochemical and biochemical techniques.
Methods
Human and bovine semen samples were processed using density gradient centrifugation (DGC). Prepared spermatozoa were either permitted or not permitted to capacitate and acrosome react in sperm preparation medium (containing or not containing calcium, albumin and $NaHCO_3$). Samples were subsequently exposed to hyaluronan conjugated with tetramethylrhodamine isothiocyanante (HA-TRITC) and to antibodies to the major HABPs (CD44 and RHAMM). Capacitation and acrosome status, respectively, were monitored using chlorotertacycline (CTC) and the Pisum sativum agglutinin (PSA-FITC). Membrane proteins extracted from capacitated sperm were also probed for HA-binding proteins after SDS PAGE and western blotting.
Results
The results indicated that two major HABPs, CD44 and RHAMM are located on the anterior acrosome and post-acrosomal sheath of non-capacitated bovine spermatozoa. In addition, these receptors became more restricted to the anterior of the acrosome and were essentially lost in capacitated spermatozoa following the acrosome reaction. However, HA-TRITC (a non-target-specific hyaluronan binding reagent) strongly labelled the sperm plasma membrane and the post-acrosomal sheath. HA-TRITC labelling was most intense on the plasma membrane of capacitated spermatozoa and was particularly strong on acrosome reacted sperm. Western blot analysis of SDS-PAGE resolved sperm membrane proteins revealed a number of bands recognized by anti-CD44 and anti-RHAMM antibodies.
Conclusion
The presence of hyaluronic acid binding proteins was confirmed in bovine and human sperm by a HA ligand or by antibodies to CD44 and RHAMM and were shown to be influenced by the cells capacitation and acrosomal status.

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P028

Expression of coxsackie virus and adenovirus receptor (CXDAR) affects tight junction complex ADN cell lineage in mouse blastocyst

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Coxsackie virus and adenovirus receptor (CXADR also known as CAR), a tight junction component molecule was reported to be expressed in epithelial cells. Previous studies reported that CXADR play an important role in tight junction complex. However, the role of CXDAR in blastocyst formation has not been investigated. Here we demonstrated that transcript levels of CXADR were elevated at eight-cell stage onward and highest at the blastocyst. To investigate the biological function of CXADR, we depleted both maternally and zygotically expressed CXADR employing an RNAi approach. Microinjection of CXADR siRNA resulted in a ~75% reduction of mRNA. In CXADR knockdown (KD) embryos, blastocyst development was significantly lower and most embryos were arrested at morula to blastocyst transition. We also found that genes required for and involved in tight junction biogenesis using qRT-PCR. Expression of tight junction component molecules including Cldn4, Ocln, and Tjp2 were decreased in the CXADR knock-down (KD) embryos. Moreover cell fate related genes such as Oct4, Cdx2, and Nanog were also downregulated in the KD embryos. In conclusion, our results suggested that CXADR affects establishment of tight junction complex and cell lineage in mouse blastocyst.
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P029

Successful isolation, culture and karyotyping of equine placental cells from failed early pregnancies

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Early pregnancy loss (EPL) in the mare is defined as loss of pregnancy between initial detection and day 65 of gestation. It occurs in 7–10% of pregnancies and yet little is known about the underlying pathologies. A lack of suitable conceptus material has limited investigation into the role of chromosomal defects in EPL. The objective of this study was to develop a method to isolate and culture placental cells isolated from failed pregnancies to enable further genetic characterisation. Conceptus material was collected from thoroughbred mares suffering an EPL by sterile uterine lavage. Conceptuses were shipped in HBSS/10% horse serum/10 μg/ml amphotericin. Tissue was isolated from the allantochorion and chorion and cells cultured at 37 °C 8% CO_2 in i) Chang D (Irvine Scientific) + amphotericin B + kanamycin sulphate, ii) AmnioChrome Plus (Lonza) + amphotericin B or iii) DMEM/10% FCS supplemented with penicillin–streptomycin and L-glutamine. Viability of cells was determined by trypan blue exclusion. Chromosome preparations were attained using colcemid and hypotonic solutions and methanol-acetic acid fixation. Twenty-nine conceptuses were submitted to the laboratory. The median gestation age EPL was detected was 42 days (range 18–65) and mean gestational age at time of uterine flush was 45.5 days (range 24–70). The median time between uterine lavage and arrival at the laboratory was 22 h (range 3.5–72). These factors did not influence the outcome of the cultures. Cells were successfully isolated and cultured from 62% of submitted conceptuses and 79% of conceptus cells selected for culture. Viable cells were more likely isolated with the use of AmniochromePlus compared to Chang D ($P=0.003$). Successful karyotyping from metaphase spreads identified from culture of one of the conceptuses showed a normal karyotype. In conclusion, we have identified a method to isolate, culture and subsequently karyotype cells derived from failed early equine pregnancies.

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P030

The endocrine disrupting chemicals bisphenol A, dichlorodiphenyltrichloroethane (DDT), methoxyclor and ethinylestradiol modulate thecal steroidogenesis *in vitro*

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Introduction
Previous reports in different species have shown that various endocrine disrupting chemicals (EDCs) can modulate ovarian steroidogenesis with the majority of studies focussing on granulosa cells. To test the hypothesis that exposure to EDCs might also perturb thecal steroidogenesis we conducted *in vitro* dose–response experiments to evaluate the direct effects of selected EDCs on androstenedione and progesterone production by cultured bovine theca cells.
Methods
Theca interna cells from 4 to 8 mm follicles were cultured for 6 days in serum-free medium. Cells were treated for 4 days under basal and LH-stimulated conditions with/without selected EDCs including bisphenol A (10 pM–10 μM) DDT (50 pM–50 μM), methoxyclor (50 pM–50 μM) and ethinylestradiol (10 pM–10 μM). Androstenedione and progesterone concentrations in media

were determined by ELISA; viable cell number was determined by neutral red uptake assay. Results are based on four independent batches of cells.
Results and discussion
Bisphenol A at 1–10 μM, DDT at 5–50 μM, and methoxyclor at 5–50 μM suppressed basal and LH-induced secretion of both androstenedione and progesterone ($P<0.001$) without affecting viable cell number. In contrast, ethinylestradiol at 1–10 μM enhanced basal and LH-induced androstenedione production ($P<0.001$) but suppressed LH-induced progesterone production ($P<0.05$). These results indicate that whilst lower (sub-micromolar) concentrations of these EDCs are without effect, at higher concentrations they can directly perturb steroidogenesis by ovarian theca cells. Studies are in progress to address the mechanism(s) through which thecal steroidogenesis is affected (e.g. altered expression and/or activity of steroidogenic pathway components). Whether environmentally-relevant exposure levels of these EDCs would generate intraovarian concentrations sufficient to adversely affect steroidogenesis remains to be established.

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P031

Does maternal progesterone supplementation in early pregnancy affect fetal development?

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Some adult diseases are programmed *in utero* by fetal exposure to abnormal concentrations of steroid hormones. Threatened miscarriage in early pregnancy is treated by progesterone in many countries although robust evidence of efficacy is lacking. We hypothesised that increased progesterone concentrations may alter fetal development. In a small pilot study, using a pregnant sheep model, we administered 200 mg progesterone twice weekly from d20–d75 of gestation and collected fetuses at d75. We examined fetal pituitary by RT-PCR, maternal and fetal serum by ELISA and fetal testis by qRT-PCR and immunohistochemistry. Maternal plasma progesterone was not increased by progesterone administration, suggesting rapid clearance. However fetuses from mothers that were supplemented by progesterone had higher progesterone concentrations compared to controls ($P=0.04$) suggesting reduced clearance and augmented fetal exposure. There was no difference in the expression of LHB and PGR in the pituitary. The expression of AR, PGR, ESR1 and WT1 in the testis did not differ between the control and progesterone treated groups. Although the small numbers precluded statistical significance, there was a trend towards increased expression of AMH, INSL3, ESR2, LHCGR and STAR in the progesterone treated group. AR immunostaining, using blinded assessment, was consistently different in progesterone treated animals. We observed increased immunostaining intensity in the Sertoli cells and reduced immunostaining in the Leydig cells when compared to controls. Progesterone given to mothers crosses the placenta and is maintained in the fetal circulation. It could therefore exhibit direct and indirect effects on the fetus via progesterone receptors, its metabolism to other steroids or their displacement from serum proteins. This pilot study suggested the possibility of fetal testicular alterations and highlights need for further research into whether there is a lifelong legacy on offspring health as a result of therapeutic maternal progesterone administration in early pregnancy.

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P032

PGF2α-PTGFR signaling promotes angiogenesis in the porcine endometrium during early pregnancy

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Introduction
Prostaglandin F2α (PGF2α) is mainly known to be involved in luteolysis. However, our recent studies indicate that PGF2α synthesis and its receptor (PTGFR) expression are up-regulated in the porcine endometrium during embryo implantation. Aims of present study were: i) to immunolocalize PTGFR protein in uterus; ii) to elucidate the involvement of PGF2α-PTGFR signaling in angiogenesis in the porcine endometrium.
Methods
Sections of uterus collected on day 12 of the estrous cycle and pregnancy were processed for immunolocalization of PTGFR protein by IHC. Effect of PGF2α on vascular endothelial growth factor (VEGFA) synthesis and secretion by endometrium was studied by incubation of endometrial explants collected from gilts ($n=6$) on day 12 of the estrous cycle with PGF2α (100 nM, 1 μM) and fluprostenol (1 μM) for 24 h. After incubation, VEGFA mRNA expression was assessed by qPCR. Concentration of VEGFA in culture media was measured by RIA. Subsequently, primary endothelial cells from porcine endometrium (EnPrim) were treated by PGF2α (100 nM) alone or incubated in the presence/absence of VEGFR inhibitor (AAL-993) with conditioned media from endometrial explants treated with vehicle or 1 μM PGF2α together/without PTGFR antagonist (AL8810). Cell proliferation was assessed by colorimetric method.
Resulst and discussion
PTGFR protein was localized mainly in luminal and glandular epithelium as well as in blood vessels. PGF2α (1 μM) and fluprostenol (1 μM) elevated VEGFA gene expression and secretion by endometrial explants ($P<0.05$). Conditioned media from PGF2α-treated endometrial explants increased ($P<0.001$) proliferation of EnPrim. This effect was abolished by blocking of PTGFR in endometrial explants as well as by blocking VEGFR in endothelial cells. No direct effect of PGF2α was observed on endothelial cells proliferation. Our results indicate indirect PGF2α-PTGFR involvement in angiogenic changes in porcine uterus by elevation of endometrial VEGFA content, followed by stimulation of endothelial cells proliferation.
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P033

Functional evaluation of miRNAs during the ovarian follicular/luteal transition

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Little is known about the involvement of miRNAs in luteal development. Cattle provide a convenient model to study ovarian physiology in monovular species. Our previous microarray studies in bovine showed significant upregulation of miR-96 and miR-132 in luteal relative to follicular tissues. In the present study we used an *in vitro* model of forskolin-induced follicular granulosa cell luteinisation, transfection with specific locked nucleic acid inhibitors or mimics of miR-132 and miR-96 led, respectively, to abolished expression and a significant increase in the levels of these miRNAs ($P<0.01$) within 4 days. The induced changes in miRNA levels during luteinisation did not have any effect on the transcript levels of predicted mRNA targets including FOXO1and ADCY6. We then investigated the functional involvement of these miRNAs in cultured bovine luteal cells. Inhibition of miR-132 and miR-96 led to abolished expression of these miRNAs ($P<0.01$) within 2 days, loss of function of these miRNAs did not have a significant effect ($P>0.1$) on progesterone production by luteal cells. However, their effects on the expression of the putative target of miR-96 and miR-132, FOXO1, were determined. Inhibition of miR-132 led to an increase ($P<0.05$) in transcript but not protein levels of FOXO1. In contrast, inhibition of miR-96 resulted in increased protein but not transcript levels of FOXO1. Our results indicate a potential involvement of miR-96 and miR-132 in promoting luteal cell survival. To confirm this, we are currently investigating the effects of these two miRNAs on apoptotic genes and the response of luteal cells to different pro-apoptotic stimuli.

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P034

Effect of cryopreservation on follicular development in human ovarian tissue

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Introduction
Recent progress in cancer therapy has significantly decreased mortality rates. However, these therapies whilst curative can cause significantly reduced fertility

or sterility. Current fertility preservation methods are limited to women who either have a partner or who will use donor sperm and without a hormone sensitive tumor. Ovarian tissue transplantation could offer an alternative for these patients. Unlike other studies, we assessed the impact of cryopreservation on follicular development in post-pubescent ovarian tissue by investigating the effects of cryopreservation on the expression of 3 proteins involved in follicular growth.
Methods
Ovarian biopsies from women undergoing cesarean section were used for fresh and cryopreserved xenografting into nude mice and left for 145 days. The mice were stimulated with gonadatropins and Ki67, AMH and FSH receptor immunostaining was carried out and quantified.
Results
Fresh biopsies produced a greater number of follicles but those from cryopreserved biopsies reached more advanced developmental stages. Expression of MIB-1 and FSH receptor staining was higher in follicles of the cryopreserved samples than in the fresh, but AMH staining was 40% greater in fresh biopsies. 37.5% of fresh biopsies developed a corpus luteum (CL) and 12.5% presented with calcification compared with 10 and 50% respectively in cryopreserved biopsies.
Conclusion
This is the first study to use FSH receptor staining to validate follicular staging and use quantitative IHC analysis. The results suggest accelerated development through to ovulation in fresh biopsies and that cryopreservation interferes with follicular growth. We postulate that ischemia is the cause of follicular developmental delay in cryopreserved tissue.

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P035

Differential gene expression in granulosa–lutein cells from women with polycystic ovaries is independent of the dose of FSH given for ovarian stimulation

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Polycystic ovary syndrome (PCOS) affects over 5% of women of reproductive age. It is associated with an ovulatory infertility, menstrual disturbances as well as metabolic abnormalities including obesity and insulin resistance. PCOS has a strong genetic basis but studies of ovarian gene expression in PCOS are limited by the difficulty in obtaining tissue samples from women with and without PCOS. For that reason, most studies have been performed in granulosa–lutein (GL) cells obtained as a by-product of egg collection for IVF. However, none of the studies to date has taken into account the effect of the dose of exogenous FSH given to stimulate multiple follicle development, an important issue considering that modern stimulation regimens typically employ lower doses of FSH for superovulation in women with PCO (Hardy *et al*., *Hum Reprod* 1995 **10** 2125–35). The aim of this study was to examine differential expression of key genes involved in gonadotrophin action and steroidogenesis in GL cells from women with normal ovaries ($n=5$) and two groups of women with PCO, those with polycystic ovary syndrome ($n=9$) and those with PCO morphology who had a regular cycle ($n=6$) using quantitative PCR. As shown previously (Catteau-Jonard *et al*., *JCEM* 2008 **93** 4456–61), AMH (threefold, $P<0.05$) and FSHR (two- to threefold, $P<0.05$) were upregulated, but a novel finding was the reduction in expression of CYP11A1 ($P<0.001$) in GL cells from both PCOS and PCO groups. Importantly, although the total dose of FSH used for superovulation in women with PCO/PCOS was typically half that in controls, there was no relationship between levels of gene expression and dose of FSH in individual subjects. In conclusion, we demonstrate distinctive gene expression profiles in granulosa cells from women with PCOS and PCO which are independent of the dose of FSH used for ovarian stimulation.

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P036

Effects of maternal peri-conception and first trimester protein supplementation on circulating progesterone levels and concomitant conception rates in yearling heifers

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Introduction
This study investigates the effect of dietary crude protein on conception in range beef heifers and uses circulating progesterone as a measure of early embryonic loss known to be a major cause of reproductive inefficiency.
Methods
Primaparous 14 month old *Bos indicus* cross heifers ($n=350$) were selected from a range population. 60 days prior to artificial insemination they were randomly assigned to two equal groups and individually fed isocalorific high (14%) or low (7%) crude protein (CP) pellet diet. Heifers underwent an 8 day progesterone based synchronisation program and, on day 0, were artificially inseminated (AI) to one bull. At 23 days post-conception (dpc) the two groups were further split into high or low % CP creating four treatment groups: i) high/high (HH), ii) low/high (LH), iii) high/low (HL) and iv) low/low (LL). Pregnancy was confirmed by rectal ultrasound at 36dpc. Plasma progesterone was assessed prior to AI and at 23 and 36dpc.
Results
Overall pregnancy rate was 35.3%. Conception rates for the heifers who received high protein diet during the peri-conception period (-60 to 23dpc) was 8% higher than those that received the low protein diet (39.55% vs 31.30%). Heifers that received the high protein diet during the peri-conception period had significantly higher circulating progesterone levels at 23 and 36dpc compared to those that received the low protein diet ($P<0.05$). A reduction in circulating progesterone has been associated with increased levels of early embryonic loss. From the laboratory assay used, 7 ng/ml of progesterone in plasma was considered indicative of pregnancy. By this measure, 27 heifers lost the embryo between 23dpc and 36dpc, 19 of these (70%) had received low protein diet during the peri-conception period.
Conclusion
Protein supplementation during the peri-conception period in range heifers may increase pregnancy rates via increased circulating progesterone and decreased early embryonic loss.
Acknowledgement
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P037

***In vitro* culture of IVM derived porcine embryos: comparisons of single-step and sequential media systems on embryo quality and cryosurvivability**

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In pigs, the quality of *In vitro*-produced embryos is much lower than that of *in vivo*-derived embryos. The poor quality of *In vitro*-produced porcine embryos has been attributed to the apparently sub-optimal embryo culture media that is commonly created in-house. Few studies have examined the capacity of commercially available media, typically used to culture human embryos, to support the development of porcine embryos. The aim of the present study was to determine the effectiveness of in-house and commercially available single-step and sequential media systems in supporting the development of *in-vitro* produced porcine embryos. Embryos were generated using standard *In vitro*-production protocols and cultured for 7 days prior to vitrification. In experiment 1 (single-step media), embryos were randomly allocated to 2 groups (in-house porcine zygote medium-3 (PZM3): $n=503$; Origio™: $n=464$). In experiment 2 (sequential media), embryos were randomly allocated to 3 groups (in-house modified PZM3: $n=239$; Origio™: $n=222$; Vitrolife™: $n=241$). Each experiment was replicated three times. In experiment 1, embryos cultured in the single step PZM3 formed blastocysts at a greater rate than those cultured in the single step Origio™ medium (33% vs 21%; $P<0.05$). While blastocysts of the Origio™ group tended to display a greater post-thaw survival rate than those of the PZM3 group (72% vs 54%; $P=0.09$). In experiment 2, embryos cultured in the three sequential media systems, modified PZM3, Origio™ and Vitrolife™,

cleaved (76%, 80 and 82%, respectively) and formed blastocysts (32%, 29% and 30%, respectively) at similar rates. Furthermore the post-thaw survival rates (67% to 69%) did not differ significantly. The results indicate that the in-house media was more effective than commercial single-step medium in supporting the development of *In vitro*-produced porcine embryos to the blastocyst stage, but there were no significant differences in embryo development or post-thaw survival rates between the in-house and commercial sequential media systems.
Key words: porcine embryo, single step medium, sequential media, vitrification
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P038

Experimental models for challenging the 'KNDy hypothesis': the acute response of GnRH secretion to nutrition

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In the "KNDy hypothesis", the latest concept explaining the control of GnRH secretion, the pulsatile GnRH signal is controlled by arcuate nucleus cells that produce kisspeptin (K), neurokinin B (N) and dynorphin (Dy). The interplay among these peptides involves one of them acting as a 'pace-setter', one as a 'brake', and the other as communicator of the final signal to the GnRH cells. To date the KNDy hypothesis has proven to be robust, but, if it is to persist, it needs to be able to explain acute changes in GnRH pulse frequency such as that evoked by metabolic signals. Metabolic status is a powerful regulator of reproductive activity – for example, an increase in food intake can stimulate testis function in male sheep. This response is mediated at least partly by an increase in GnRH pulse frequency that begins within a day of nutritional supplementation. For this response to offer an experimental model for challenging the KNDy hypothesis, we need a more precise measure of the rapidity of the increase in GnRH secretion after feed supplementation. We therefore studied sexually mature rams ($n=24$) that were acclimated to a diet designed to maintain constant bodyweight. On the day of experimentation, half the rams were given a supplement (150 g lupin grain). Blood was sampled from 8 h before feeding until 11 h after feeding. In supplemented rams, LH pulse frequency increased from 0.81 ± 0.277 pulses per 8 h to 3.30 ± 0.464 pulses per 8 h ($P<0.001$). In control rams, there was no change in pulse frequency (0.75 ± 0.250 vs 1.95 ± 0.644 pulses per 8 h; $P>0.10$). We have now begun using this model to test whether the KNDy cells are involved in nutrition-induced increases in frequency of GnRH pulses.
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P039

Poster withdrawn.
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P040

Sex specific effects of maternal dietary protein during the periconceptional period upon hepatic gene expression in heifer progeny

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Sex specific expression occurs in 20–30% of all hepatic genes in the adult animal (1). We have shown that there is an interaction between gender and the level of maternal dietary protein intake on bovine fetal development as early as 36–39 days post conception (dpc) We hypothesise that exposure to different levels of maternal protein intake in the periconceptional period (60d preconception to 23dpc) and in the postconceptional period (23–98dpc) will result in a differential pattern of gene expression in the male and female fetal bovine liver at 98 dpc. We found that high protein intake resulted in an increase in the hepatic expression of the glucocorticoid receptor (GR) ($P<0.01$) in the female fetus only. Similarly a high protein intake in the periconception period resulted in an increase in the hepatic expression of the gluconeogenic factors, PEPCK-C ($P=0.05$), PGC-1α ($P<0.05$) and PDK-1($P<0.05$) in female. Low protein given periconceptionally effected increased expression of genes permissive to lipogenesis; PPARγ ($P<0.01$) and RXR ($P<0.01$) in the female liver. The expression of Fox01, a transcription factor with a key role in insulin signalling and gluconeogenesis, was increased in both sexes after exposure to a high protein diet in the periconceptional period. Our findings highlight the sex specific sensitivity of the epigenome to maternal nutrition around conception and very early gestation. Furthermore, such effects may have functional consequences in the decreased sensitivity of the hepatic insulin signalling pathway (2).
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P041

Regulation of CyclinD2 by Smad3 and Foxl2 during early follicle development

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Introduction
Primordial follicles are relatively quiescent structures that form the basis of the ovarian reserve. Maintenance of the quiescent state, and conversely, release from this state towards irreversible growth, involves mechanisms that are currently unresolved. Two transcription factors have been implicated alongside this process in granulosa cells (GCs). Specifically, Smad3 was recently identified in the nuclei of GCs in small single-layered follicles whereas Foxl2 is a fundamental regulator of GC viability and phenotype. Since Smad3 interacts with Foxl2 in other models, we aimed to determine whether these two transcription factors co-operate in GCs to directly regulate the cell cycle regulator CyclinD2.
Methods
To study the protein expression pattern of Smad3, Foxl2 and CyclinD2 throughout the different follicle stages, immature mouse ovaries (containing high proportions of small follicles) were immuno-fluorescently labelled and imaged by high-resolution confocal microscopy. Follicles were staged and >3700 GC nuclei were analysed using Image J. A proximity ligation assay (PLA) was used to visualise a direct interaction between the two proteins. In parallel, samples of whole ovaries were used for ChIP-PCR and qPCR to determine if Foxl2 and Smad3 directly bound to the gene promoter of *CyclinD2 in vivo*.
Results and discussion
Smad3, Foxl2 and CyclinD2 proteins co-localised in GC nuclei and followed a similar pattern of expression in small follicles. Specifically, staining intensity increased in transitional and primary stages, followed by a reduction in expression as follicles formed multiple layers. In addition, *CyclinD2* promoter fragments were detectable in Foxl2 and Smad3-bound chromatin complexes in neonatal ovary samples. Together these findings suggest that both transcription factors are regulating CyclinD2 in a co-operative manner; however, a direct interaction was not evident by PLA. The relationships presented here further implicate the TGFβ pathway during follicle activation, where recruitment of cofactors may be important for signalling in this context.
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P042

Genome-wide cDNA microarray screening to correlate gene expression profiles of aged mouse ovary to the size of the reserve of the primordial follicle

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Size of the reserve of primordial follicles in mammalian ovary is critical for female reproduction and reproductive senescence. Despite an accurate estimation of the size of the reserve of primordial follicles remains unestablished, due to lack of molecular markers. We performed to explore the expressed gene in the ovarian correlated with primordial follicle in posterior reproductive duration using a wide array in mouse. ICR mouse bread until 9 or 58 weeks was used (9W and 58W). 58W mice were divided into three groups: administrated vehicle, carnitine

(5 mg/ml) or saturated hydrogen molecule (H_2) in drinking water. Mice were treated superovulation by PMSG and hCG, and in one side of ovary was fixed to count primordial follicle and another one was used for analysis for gene expression by real time PCR and microarray. The number of ovulated oocytes in all groups of 58W mice decreased significantly and increased number of abnormal ova in comparison of 9W. The number of primordial follicle in aged mice ovary varied between the individual mice. Although controlling the reserve of primordial follicles related gene, Foxo3, PTEN and Akt2 were expressed in 9W and 58W mice ovary, there was no significant difference between 9W and 58W. The gene expression in aged mice ovary correlated with its size of primordial follicle was analyzed using microarray. Gstp2, Gapdh, Msmo1, Actb (β-actin) and Akr1b7 gene revealed correlation with the size of primordial follicle ($P<0.05$). Microarray analysis was revealed that the size of primordial follicle was correlated the constitutive genes required for the maintenance of basic cellular function.

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P043

Melatonin-induced regulation of vascular endothelial growth factor (VEGF) isoform expression and microvasculature remodelling in the pituitary gland of seasonally anoestrous ewes

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Recent studies have shown that microvasculature remodelling of the ovine pituitary gland is seasonally regulated through mechanisms involving the differential expression of pro-angiogenic ($VEGF_{165}$) and anti-angiogenic ($VEGF_{165b}$) isoforms of vascular endothelial growth factor (VEGF). As for other photoperiodic species, the pattern of pineal melatonin secretion mediates the effects of day length on the sheep seasonal reproductive cycle. In this study, we investigated whether timely administered melatonin can alter the pituitary microvasculature and VEGF isoform expression in seasonally anoestrous ewes exposed to natural photoperiod. Melatonin (1.5 mg/dose, i.m.) was injected twice daily at 0500 and 1530 h for 18 days during the spring; control animals were injected with vehicle ($n=4$/group). The effects of treatment on the pituitary microvasculature and on the expression of VEGF isoforms in the pars tuberalis (PT) and pars distalis (PD) were investigated by immunohistochemistry. Melatonin reduced the number of vascular loops connecting the PT with the infundibulum ($P<0.05$). In both the PT and PD, the expression of $VEGF_{165b}$ was significantly increased in melatonin-treated sheep ($P<0.001$). In these animals, but not in controls, $VEGF_{165b}$ was more abundant in the PT than in the PD ($P=0.01$). No difference in $VEGF_{165}$ expression was observed between groups in the PT. However, in the PD, $VEGF_{165}$ was higher in control ewes ($P<0.001$). Critically, the aforementioned melatonin effects were concomitant with a robust up-regulation of GnRH expression in neuronal terminals of the median eminence ($P<0.001$). Collectively, these findings show that the previously reported seasonal control of VEGF expression in photoperiodic species is mediated by melatonin. Specifically, the increased duration of the nocturnal melatonin rise during short days up-regulates the anti-angiogenic $VEGF_{165b}$ isoform and restricts the vascular connection between the PT and the infundibulum as part of the mechanisms underlying the photoperiodic control of the annual reproductive cycle.

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P044

Melatonin regulates seasonal variations in prolactin and follicle stimulating hormone synthesis via alternative splicing of pituitary vascular endothelial growth factor-A

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The pars tuberalis (PT) of the ovine pituitary contains high-density melatonin receptors (MT1) known to participate in the seasonal regulation of prolactin (PRL) output from lactotroph cells in the pars distalis (PD). However, the paracrine mechanism relaying the signal from the PT to the PD remains unresolved. We have recently shown that MT1-positive cells in the PT co-express vascular endothelial growth factor-A isoforms ($VEGF_{165}$/$VEGF_{165}$b), and that VEGF-receptors are expressed in the PD. In this study, individual primary PT and PD cell cultures were generated from ewes during the breeding season (BS) and non-breeding season (NBS). PT cells were treated with: i) media (24 h); ii) BS-like melatonin (16 h:8 h, melatonin:media); iii) NBS-like melatonin (8 h:16 h melatonin:media). Treatments were applied to match the nocturnal rise of endogenous melatonin. PD cells were treated with: i) media (24 h); ii) BS-like $VEGF_{165}$ (16 h:8 h, $VEGF_{165}$:media); iii) NBS-like $VEGF_{165}$ (8 h:16 h, $VEGF_{165}$:media); iv) NBS-like PT-conditioned media; v) BS-like PT-conditioned media; vi) melatonin; vii) TRH; viii) NBS-like conditioned media?anti-$VEGF_{165}$ antibody; and ix) BS-like conditioned media?anti-$VEGF_{165}$b antibody. Cell lysates were obtained after 6 days in culture for quantification of PRL and FSH mRNA by QPCR.
In PT cultures, BS-like melatonin treatment up-regulated $VEGF_{165}$b during both BS and NBS. Conversely, treatment with NBS-like melatonin caused an up-regulation of $VEGF_{165.}$ Responses to BS and NBS-like melatonin regimens were greatest when melatonin matched the season of application.
In PD cultures, NBS-like $VEGF_{165}$ increased PRL expression, whilst BS-like $VEGF_{165}$ up-regulated FSH. NBS-like conditioned media (NBSCM) resulted in a greater increase in PRL than $VEGF_{165}$ alone. However, NBSCM?anti-$VEGF_{165}$ abolished the NBSCM-mediated PRL rise. Similarly, BS-like conditioned media (BSCM) caused a larger increase in FSH than $VEGF_{165}$ alone, which was blocked when BSCM + anti-$VEGF_{165}$b was applied.
These results reveal that VEGF-A isoforms are involved in relaying the photoperiodic signal from MT1-PT cells to endocrine cells of the ovine PD.

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P045

Periovulatory oxygen levels within the porcine oviduct obtained by laparoendoscopic single-site surgery

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This work was aimed at determining *in vivo* levels of oxygen (O_2) within the porcine oviduct. 13 gilts (G) and eight sows (S) were used. A left lateral paralumbar laparoendoscopic single-site surgical approach (GelPOINT Advanced, SingleMedical→) was carried out under CO_2 pneumoperitoneum. Laparoscopy manoeuvres allowed pulling up the ovary towards the single-site port and upon visual inspection pigs were assorted into preovulatory (PreO) or postovulatory (PostO) stages. A luminescent O_2 probe coupled to a register unit (Al300, Neofox, Oceanoptics→) was sequentially inserted into the ampulla (Amp) and isthmus (Isth) for a time period of 8–10 min after signal stabilization. Registers were obtained after replacing back the organs -with the probe inserted- into the abdominal cavity. Moderate hypoxia levels were observed within the porcine oviducts (39–85.8 mmHg or 5.13–11.29%). O_2 levels were significantly different for the maturity state (79.0 ± 10.6 vs 51.7 ± 8.4 mmHg for G and S respectively, $P<0.001$) and the region of the oviduct (71.4 ± 16.7 vs 64.6 ± 16 mmHg for Amp and Isth respectively, $P<0.001$). While the phase of the estrous cycle did not directly affect the O_2 levels (66.1 ± 14.4 vs 71.4 ± 18.2 mmHg for PreO and PostO respectively, $P=0.36$), an interaction between the phase of the estrous cycle and the region of the oviduct was observed ($P<0.001$). The reduced O_2 content within the pig oviduct is consistent with previous results in other mammals. The particular effect of the pig maturity and the oviduct region on the O_2 levels (higher values in G and Amp) may contribute to a better understanding of the role of O_2 on the physiology of the oviduct and should be considered for assisted reproductive techniques. Work supported by project AGL2012-40180-C03-03 (MINECO, Spain).

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P046

Effect of *Rhodobacter sphaeroides* LPS on toll-like receptor 4 in bovine endometrial cells

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Introduction
Endometritis is one of the most common reproductive diseases affected postpartum bovine uteri. Lipopolysaccharide (LPS) from Gram-negative bacteria

is recognized by Toll-like receptor 4 (TLR4). Binding of LPS and TLR4 mediates the expression of proinflammatory cytokines and chemokines. The excessive inflammatory responses could be involved with endometrial damage.TLR4 antagonist *Rhodobacter sphaeroides* LPS (RsLPS), is a potent antagonist of LPS from pathogenic bacteria. This study aimed to assess the effect of TLR4 antagonist RsLPS on the responses of bovine endometrium.
Methods
Primary cultures of mixed endometrial epithelial and stromal cells were challenged with LPS (100 ng/ml) after treated with and without TLR4 antagonist (5000 ng/ml). The mRNA expression of TLR4, TNFα and CXCL8 (also known as IL8) were investigated using quantitative real-time PCR.
Results and discussion
As expected, LPS up-regulated the expression of TNFα and CXCL8 ($P<0.01$). Endometrial cells treated LPS following the exposure of RsLPS showed significantly increased TLR4 expression ($P<0.05$). On the other hand, LPS did not significantly enhanced mRNA expression of TNFα and CXCL8 ($P>0.05$) in the endometrial cells pre-treated with RsLPS. Our findings suggested that the TLR4 antagonist may act as a potential treatment of clinical endometritis by mainly competition with endotoxin LPS for the same binding site on extracellular protein of TLR4 complex.

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P047

Waddlia chondrophila stimulates CXCL8 expression in ruminant trophoblast cells via p38 and p42/44 MAPK dependent pathways

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Background
Waddlia chondrophila (*W. chondrophila*) is an emerging abortifacient pathogen which has been identified in the placentae of humans and cattle. The organism is a member of the order *Chlamydiales*, and shares many similarities at the genome level, and in growth studies, with other well-characterised zoonotic chlamydial abortifacients, such as *Chlamydia abortus* (*C. abortus*). We have previously observed significant responses in the expression of pro-inflammatory mediators including CXCL8 following active infection with *W. chondrophila*. This study investigates the growth of the organism together with the signalling pathways responsible for CXCL-8 release in a ruminant placental cell line.
Methodology/Principal findings
Using qPCR, fluorescent immunocytochemistry and electron microscopy, we characterised the infection and growth of *W. chondrophila* within the ovine trophoblast AH-1 cell line. Inclusions were visible from 6 h post-infection (p.i.) and exponential growth of the organism could be observed over a 60h time-course. CXCL-8 release was significantly elevated in AH-1 cells at 24 h p.i. by active-infection with live *W. chondrophila* but not by exposure to UV-killed organisms. Chloramphenicol treatment of infected cells reduced, but did not completely ablate, CXCL-8 release indicating that active infection and organism growth are key stimuli for CXCL-8 release. MAPK signalling pathways have been demonstrated to be central to chlamydial induction of CXCL-8 in epithelial cells, and in this study we have now demonstrated that *W. chondrophila* induced phosphorylation of both p38 and p42/44 MAPK. Inhibition of either of these pathways significantly reduced the release of CXCL-8 in response to infection.
Conclusions/ significance
The abortifacient pathogen *W. chondrophila* actively infects and replicates within ruminant trophoblast cells, stimulating CXCL-8 release. Release of CXCL-8 is significantly reduced by inhibition of either p38 or p42/44 MAPK, indicating a key role for this pathway in the innate immune response to infection with *W. chondrophila*.

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P048

The effect of prenatal exposure to androgen and to a high fat diet on obesity in female mice

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Polycystic ovary syndrome (PCOS) is a complex endocrine disorder that presents in women of reproductive age. The aetiology of PCOS is poorly understood, however it is likely that women with this disorder are predisposed to produce excess androgen at or well before puberty. Environmental and lifestyle factors then contribute to the multifarious symptoms observed. One such key external factor is diet. These studies utilised a prenatally exposed androgen mouse model to investigate i) the effect of foetal exposure to excess androgen and ii) the effect of a high fat modern diet, on factors and mechanisms that underpin PCOS and determine its severity.
Pregnant C57BL/6 mice were given daily subcutaneous injections of 250 μg DHT or sesame oil on days 15, 16, and 17 of gestation. At weaning, female pups from each DHT/control litter were randomly separated into cages with either standard chow or a 60% high fat diet and maintained for 6 weeks. Animals and food intake were monitored weekly and at the end of the study glucose tolerance tests were performed after an overnight fast before animals were sacrificed and tissues and plasma were harvested.
Prenatally androgenised mice fed a high fat diet gained significantly more weight than litter mates on a standard chow diet or control mice, on either diet ($P<0.01$). Mice fed a high fat diet were glucose intolerant compared to mice fed a normal chow diet ($P<0.0001$) but prenatal androgenisation alone did not affect blood concentrations.
In conclusion, this study provides strong evidence to support the notion that exposure to excess androgen during early development predisposes to metabolic characteristics of PCOS. Furthermore, a high fat diet is a key interacting factor that results in even greater weight gain and obesity than either prenatal androgen exposure or a high fat diet alone.

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P049

Does kisspeptin exert a local modulatory effect on ovarian steroidogenesis?

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Introduction
Kisspeptin, a neuropeptide secreted in the hypothalamus and encoded by the Kiss-1 gene, has a role in promoting the release of GnRH and LH in various species. However, the possibility that kisspeptin exerts additional 'peripheral' actions at the level of the bovine gonad has not been investigated. The current aims were to investigate whether: i) kiss-1 and its receptor (GPR54) are expressed in the bovine ovary; ii) kisspeptin or kisspeptin antagonist can modulate ovarian steroidogenesis by cultured theca (TC) and granulosa (GC) cells.
Methods
GC ($n=38$) and TC ($n=43$) 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. Total RNA was harvested for qPCR analysis and data were analysed using the ΔΔCT 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 4 days with Kisspeptin-10 (10^{-10}–10^{-6}M), Kisspeptin antagonist (K234; 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 3-6 independent cultures.
Results and discussion
Kiss-1 and GPR54 transcripts were detected in all TC, GC and CL samples with significant differences between follicle categories ($P<0.001$) and CL stages ($P<0.05$). However, TC/GC culture experiments using kisspeptin or its antagonist offered no evidence to support the hypothesis that kisspeptin has a direct intra-ovarian role to modulate follicular or luteal steroidogenesis or cell proliferation/survival.

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P050

Immunohistochemical detection of hypoxya markers in the porcine oviduct

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This work was aimed at localizing and evaluating the expression of endogenous hypoxia markers in the porcine oviduct. Oviduct samples were obtained from

reproductive tracts of sows (n = 20) in the slaughterhouse. Upon visual inspection of the ovary the samples were allocated into late follicular (LF), early luteal (EL) and late luteal (LL) phases of the estrous cycle. Ampulla and isthmus sections were stained against the following specific primary antibodies: HIF2α, VEGF, Flt-1, Flk-1, Glut-1 CAIX. Staining intensity was quantified in the epithelium, lamina propria and muscular layer. All the tested hypoxia markers showed positive immunostaining although the staining intensity showed variations according to the histological layer, the phase of the estrous cycle and, to a less extent, the portion of the oviduct. HIF2α, VEGF, Flt-1, Flk-1, and CAIX were preferentially located in the epithelium and poorly expressed in the lamina propria, while Glut-1 showed broad staining in the three histological layers. HIF2α, VEGF and its receptor Flt-1 showed maximum expression through the periovulatory stage (LF and EL phases), while no periovulatory association was observed for Glut-1. Regional variation between the ampulla and isthmus was found for HIF2α, VEGF and its receptor Flt-1 with a higher staining intensity in the ampulla at the LL phase. The ubiquitous expression of the hypoxia markers indicates that oxygen levels are critical for the regulation of the oviduct physiology. The immunohistochemical association between HIF2α, VEGF and its receptor Flt-1 could be directly involved in such a regulatory role. Additional studies involving molecular techniques, other markers such as HIF1α or pimonidazole, and direct measurement of oxygen levels are required to fully understand the potential association between hypoxia and the reproductive functions of the oviduct.

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P051

SPRASA knockout mice are sub-fertile

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Introduction

SPRASA is a protein, that is expressed in the acrosome of sperm, as wel as on the oolemma of many mammals, which we identified as the target of antisperm antibodies from some infertile men. In order to further understand the importance of SPRASA we produced knockout mice and examined the effect of this gene knockout on murine fertility.

Methods

With ethics approval (AEC_R811) knockout mice were generated lacking the expression of exons 4 and 5 of the SPACA-3 gene that encodes SPRASA, and a breeding colony established. Sperm extracted from the distal vas deferens or cauda epididymis of eight week ($n=5$) and eight month ($n=4$) old knockout or control males was counted and graded for motility. Right ovaries from control ($n=4$) or SPRASA knockout ($n=4$) females were sectioned and the number of follicles at each stage counted. Homozygous SPRASA knockout ($n=16$) or control ($n=7$) breeding pairs were monitored for litter size and time between litters.

Results

There were no significant differences in sperm count or motility between SPRASA knockout or controls ($P>0.05$) at eight weeks or eight months age. There was no significant difference in the number of follicles, at any stage of development, between control and SPRASA knockout females ($P<0.05$). Litter size was significantly (P,0.0001) smaller in SRPASA knockout (five pups) than control (seven pups) controls. Average time between litters was significantly ($P=0.0076$) longer in SPRASA knockout (32.4 days) than control mice (24.5 days).

Discussion

Our observations of mice genetically deficient in SPRASA suggest that deletion of SPRASA does not affect production of either sperm or oocytes but does reduce fertility. This result supports existing evidence from *in vitro* models showing that SPRASA is involved in sperm-oocyte interactions and confirms that SPRASA is potentially a useful target for the control of fertility.

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P052

The effect of reproductive ageing on chromatin configuration and amino acid metabolism in germinal vesicle staged sheep oocytes *in vitro*

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Reproductive ageing in females is associated with reduced oocyte maturation potential and developmental competence. Oocyte chromatin configuration and metabolism, have recently been established as correlates of oocyte developmental competence in a number of species. Here, we have evaluated the effect of reproductive ageing on oocyte chromatin morphology and amino acid turnover in germinal vesicle (GV)-staged, ovine oocytes.

Cumulus–oocyte complexes were harvested from follicles >2 mm diameter from abattoir-derived adult ($n=133$) and prepubertal ($n=135$) sheep ovaries. Denuded GV oocytes were cultured individually in 1 μl drops of serum-free IVM medium containing 50 μM cilostamide for 6 h (t6) at 38 °C and 5% CO_2. Chromatin morphology was assessed by fluorescent microscopy at time 0 (t0) and t6 after staining in 10 μg/ml DAPI. Amino acid metabolism was assayed in spent culture media using HPLC.

Chromatin morphology was significantly different in GV oocytes from prepubertal vs adult sheep ($P<0.05$). At t0, no difference in chromatin morphology was observed; most oocytes contained net-like, uncondensed chromatin (58.8% adult vs 70.4% prepubertal; $P>0.05$). At t6, significantly more adult oocytes (60.4%, $n=53$) than prepubertal oocytes (26.5% $n=55$, $P<0.05$) had progressed to the clumped chromatin configuration associated with condensation before GVBD. Amino acid profiling revealed adult oocytes consumed more isoleucine ($P=0.001$) and leucine ($P=0.026$), and produced more glutamic acid ($P<0.001$) and alanine ($P<0.001$), but less tyrosine ($P<0.001$) than prepubertal oocytes. Aspartic acid metabolism switched from low production to consumption as age increased.

In conclusion, reproductive ageing affected both amino acid metabolism and the dynamics of chromatin configuration in GV-staged sheep oocytes *in vitro*.

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P053

Investigating the action of $1,25(OH)_2D_3$/vitamin D_3 in the human ovary: relevance to fertility and polycystic ovary syndrome (PCOS)

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In recent years it has become apparent that vitamin D3 (VD) has a fundamental role in reproductive function, with deficiency of the hormone being implicated in several reproductive pathologies: endometriosis, pre-eclampsia and PCOS. VD is essential for oestrogen synthesis in both males and females; via indirect mechanisms of calcium homeostasis but also direct regulation of aromatase expression. Women with PCOS are more likely to suffer from VD deficiency, with an inverse correlation between serum VD concentrations and BMI/insulin resistance. VD supplementation has been shown to improve ovulation frequency and insulin sensitivity, indicating its significant role in ovarian steroidogenesis, ovulation and fertility. The aim of our study was to investigate VDR mRNA expression in human ovarian tissue (using qPCR), and the effect of VD on aromatase mRNA expression and promoter II (PII) activity (using a luciferase assay) in the human KGN cell-line. VDR mRNA was found in cortex and stroma of normal and PCO tissue, with higher levels in PCO cortex. VDR mRNA levels were significantly up-regulated in theca from follicles 7–12 mm compared to 5–6 mm; and was more pronounced in PCO compared normal ovaries. This expression pattern of VDR highlights the importance of VD in antral follicle progression. KGN cells were cultured with forskolin (to stimulate cAMP) ± VD at 0.02, 0.2, 2 and 20nM, with testosterone (5×10^{-7} M) as an aromatase substrate for 48 h. Cells were also transfected with PII-specific luciferase reporter construct. As expected, forskolin up-regulated aromatase mRNA expression 50-fold and interestingly VD reduced this stimulation by half but only at the two lowest doses of VD used. This was a direct effect on PII activity. VD serum levels <50 nM indicate deficiency, with severe deficiency at <12.5 nM. Hence our data showing substantial reduction of cAMP-driven aromatase expression at low VD doses, indicate that VD deficiency could contribute to the impaired folliculogenesis/ovulation identified in women with PCOS.

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P054

Characteristics of bovine granulosa cells cultured under low and high oxygen tensions in the presence of different concentrations of melatonin

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Previous studies have assessed the effects of melatonin on cultured granulosa cells under 5% CO_2 in air, and focused on its antioxidant properties. Effects of melatonin under more physiological oxygen tensions are not known. Here we cultured granulosa cells from antral follicles (4–6 mm diameter) in fibronectin-coated 12-well plates (at 600 000 cell/ml) in TCM199, with melatonin added at one of four concentrations (0, 20, 200, 2000 pg/ml) under either i) low oxygen (5% O_2, 5% CO_2) or ii) in air (with 5% CO_2) for 48, 96 and 144 h. Cell viability (using trypan blue and crystal violet) and reactive oxygen species (ROS) generation (using nitroblue tetrazolium) were assessed. Mean number of viable cells was greater ($P=0.04$) under 5% O_2 than air (658 000 vs 610 000 cells). Cell number was further increased ($P<0.001$) with added melatonin under both 5% O_2 and air. ROS generation was similar under both 5% O_2 and air, but was reduced ($P=0.002$) with the inclusion of melatonin (at all levels). Oxygen tension interacted ($P<0.001$) with time in culture to affect media E2 concentration. E2 (pg/10^5 cells) under air vs 5% O_2 =4218 vs 1851 at 48 h; 858 vs 669 at 96 h; and 838 vs 2009 at 144 h. P4 (ng/10^5 cells) increased ($P<0.001$) between 48 and 96 h of culture, and was greater ($P<0.001$) at these time points under air than under low O_2 (176.5 vs 100.0). E2:P4 ratio fell between 48 and 96 h under air and remained low to 144 h (0.092, 0.005 and 0.007), whereas E2:P4 ratio under low O_2 initially fell and then increased (0.060, 0.007 and 0.020) ($P<0.001$). Cell number and viability is enhanced, luteinisation reduced and steroidogenesis altered under low O_2. Melatonin enhances cell viability irrespective of oxygen tension, but has no effect on steroidogenesis.

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P055

Impact of maternal age on oocyte amino acid turnover and mitochondria DNA copy number in sheep

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Introduction
Reproductive ageing is associated with a reduced ovarian reserve and suppression of oocyte developmental competence (quality) as evidenced by increased meiotic segregation errors, epigenetic alterations and reduced fertilisation rates. Metabolic indicators of oocyte quality such as oocyte amino acid profile (AAP) and mitochondria DNA (mtDNA) copy number may provide insights into the mechanism of declining oocyte quality with age. This study investigated the effect of adult age on oocyte AAP and mtDNA copy number in sheep.
Methods
Ovaries were collected from young (9–12 months, $n=6$), and old (6–8 years, $n=6$) Greyface ewes. Cumulus–oocyte complexes from 2 to 10 mm diameter follicles underwent individual maturation for 18 h in serum-free IVM medium before denudation and culture for 6 h in AAP assay medium. Oocyte maturity was assessed before and after AAP incubation. Amino acid turnover was measured in spent AAP assay medium by HPLC; mtDNA copy number was quantified in individual oocytes by real-time PCR.
Results
A total of 57 follicles (9.5 ± 0.6/animal) and 64 follicles (10.7 ± 1.4/animal) were collected from young and old ewes, respectively. Age had no significant effect on the capacity of oocytes to mature to MII *in vitro* (young: 91.7% MII, $n=48$ vs old: 94.2% MII, $n=52$; $P>0.05$). AAP revealed significantly ($P<0.05$) lower depletion of glutamine and leucine, as well as lower appearance of tyrosine, higher appearance of glutamic acid and lower overall amino acid depletion from/to the media of young MII oocytes ($n=42$) compared to old counterparts ($n=48$). Oocyte mtDNA copy number was significantly higher ($P<0.01$) in young ($2.4\pm0.4\times10^6$, $n=24$) vs old oocytes ($1.6\pm0.4\times10^6$, $n=24$).
These results suggest that oocyte nuclear maturation capacity *in vitro* is not affected by adult age. In contrast, the observed increase in amino acid depletion and decline in mtDNA copy number with age may be related to oocyte quality.

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P056

Serine/threonine kinase receptor associated protein (Strap) inhibits early follicle development in mouse ovaries

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Introduction
The molecular mechanisms involved in regulating growth of small, gonadotrophin-independent follicles are poorly understood. We have previously shown that the canonical TGFβ signalling intermediate, Smad3 is highly expressed in granulosa cells (GCs) of single-layered follicles. Furthermore, a reduction in the overall expression of Smad3 is associated with the onset of multi-layering and increased granulosa cell proliferation, suggesting modulation of TGFβ signalling is an important molecular event as follicles initiate growth. Strap has been identified in other tissues as an intracellular TGFβ receptor antagonist; therefore, we aimed to determine the expression and role of this protein in the context of early follicle development.
Methods
Gene and protein expression of Strap and Smad3 were analysed by qPCR and immunofluorescence/confocal microscopy in mouse ovaries at 4, 8 and 16 days of age (d4–16). Using an established culture model, neonatal (d4) mouse ovary fragments were used to examine the effects of Strap mRNA knock down using siRNA, and Strap protein inhibition by immuno-neutralisation. Effects of these treatments were compared with control groups after 7 days by measurements of oocyte size and immuno-fluorescent labelling of anti-Mullerian hormone (Amh) as a marker of GC development.
Results and discussion
Strap and *Smad3* mRNA revealed similar patterns of expression levels, being highest in d4 ovaries containing mainly primordial follicles, and significantly reduced in d16 ovaries containing many multi-layered preantral follicles. Despite the reduction in transcript expression, both proteins were weakly detectable in GCs of multi-layered follicles, where they co-localised. Interestingly, mean oocyte diameters were significantly increased in ovary fragments cultured with either Strap siRNA (28.2 μm vs 24.2 μm; non-targeting control) or Strap antibodies (31.4 μm vs 28.5 μm; IgG control) after 7 days. In the latter experiment, Amh staining was increased in the anti-Strap group relative to control, supporting an overall inhibitory role for Strap on early follicle development.

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