Little embryos do make big decisions

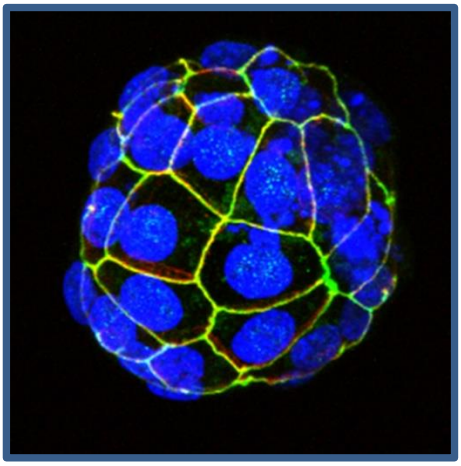
Symposium to celebrate the career of

Professor Tom Fleming FRCOG

on the occasion of his retirement

Friday 7 July 2017

St. Mary's Stadium, Southampton, UK



FINAL PROGRAMME AND BOOK OF ABSTRACTS

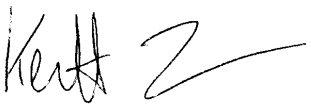
Welcome

On behalf of the local organising committee I would like to welcome you to this one day symposium **Little embryos do make big decisions.** We hope you find the science stimulating and the day rewarding.

The symposium aims to celebrate the distinguished career of Professor Tom Fleming, Professor of Developmental Biology, who has worked at the University of Southampton since 1998 and is retiring this Summer. Tom was the lead author on a Reproduction Fertility and Development review title from which the symposium gets its name: "Do little embryos make big decisions? How maternal dietary protein restriction can permanently change an embryo's potential, affecting adult health." We hope you join us in taking this opportunity to celebrate this event, and to wish Tom a very happy and long retirement with his wife Diana, family and friends present.

The symposium brings together some really excellent speakers who take us on the journey of early gamete development, and demonstrate the increasingly recognised connection between early life events and adult health. The University of Southampton is very much known as a world leading centre in Developmental Origins of Health and Disease (*DOHaD*). It is home to the MRC Epidemiology Resource Centre, and has over 100 research scientists discovering how interactions between the genome and the environment, in utero and during infancy, influence susceptibility to common diseases in adult life.

We would like to acknowledge the financial support of the Faculty of Natural and Environmental Sciences at the University of Southampton, and especially its Dean, Professor Rachel Mills. We also gratefully acknowledge the Society for Reproduction and Fertility (SRF), who have very generously sponsored this symposium. It is particularly pleasing that **Little embryos do make big decisions** is the first SRF sponsored symposium, and we hope that the society continues to support UK based reproductive biology research in this way.



Professor Keith T. Jones
Professor of Cell Biology and Head of Biological Sciences
University of Southampton

Local Organising Committee

Dr Francesca Houghton Dr Judith Eckert Dr Neil Smyth

Professor Tom P Fleming FRCOG

Tom Fleming is Professor of Developmental Biology within Biological Sciences at the University of Southampton, UK. Tom graduated in Zoology from University of Wales (1972), obtained his PhD from Polytechnic of the South Bank (University of London; 1979) for research in invertebrate reproduction and pollution, was a postdoc at University of Keele until 1981 and then Senior Research Associate at Cambridge University where he moved into the field of mouse embryology. He relocated to Southampton in 1988 as Lecturer and became Professor in 1998.

Tom's work at Cambridge with Prof Martin Johnson and continuing in early years at Southampton concerned the cell biological mechanisms of mouse embryo development including cell polarity, epithelial differentiation and blastocyst morphogenesis. His group more recently has focused on the influence of environment around the time of conception on subsequent development and adult health. Tom's pioneering studies have demonstrated that poor maternal diet (in vivo), maternal sickness (in vivo) or IVF-related culture conditions (in vitro) before the embryo implants into the uterus provoke changes in the developmental programme affecting fetal growth and postnatal disease risk, especially cardiovascular, metabolic and neurological dysfunction. His research, influenced by Prof David Barker's epidemiological studies at Southampton on disease origin, comprise a range of molecular, epigenetic, cellular and physiological technologies to help understand mechanistically the legacy of early embryonic environment on health over the lifetime.

Tom has been Editor-in-Chief of *Reproduction* from 2008-end 2012, is an editorial board member for several reproductive/developmental biology journals, is a Council member and Treasurer of the Society of Reproduction and Fertility (SRF), and sits on various grant committees and advisory boards. He has published over 150 original research publications. At Southampton, he was former Head of Cell Sciences department and Associate Dean for Research. He was made an Honorary Fellow *ad eundem* of the Royal College of Obstetricians and Gynaecologists in 2013 and was awarded the Marshall Medal from the SRF in 2013 for outstanding contributions to the study of fertility and reproduction. More importantly, he is an avid Saints supporter.

UNIVERSITY OF Southampton

S R F Society for Reproduction and Fertility

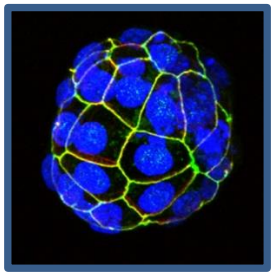


Image of a blastocyst embryo taken by Bhav Sheth, University of Southampton

Programme - Little embryos do make decisions

0930-0955	**Refreshments on arrival**
0955-1000	**Welcome** **Dr Neil Smyth** University of Southampton
1000-1030	Regulation of early ovarian follicle development **Professor Kate Hardy** Imperial College London
1030-1100	Keep Calm and Carry On **Professor Henry Leese** Hull York Medical School
1100-1130	Metabolic profiling in embryos **Professor Bernd Fischer** Martin-Luther-Universität Halle-Wittenberg, Germany
1130-1200	**Refreshment break and poster session**
1200-1230	When males become pregnant.... **Professor Alireza Fazeli** University of Sheffield
1230-1300	Placental Programming: Vulnerable trophoblast **Professor Kent Thornburg** The Moore Institute, Portland, USA
1300-1400	**Lunch**
1400-1430	Building the mammalian embryo - how to achieve a perfect partnership? **Professor Magdalena Zernicka-Goetz** University of Cambridge
1430-1500	Is spending the first 5 days of life in a test tube good for your health? **Professor Daniel Brison** University of Manchester
1500-1530	Early embryo environment and long-term offspring health **Dr Adam Watkins** Aston University
1530-1600	One-carbon metabolism: linking nutritional biochemistry to epigenetic programming of long-term development **Professor Kevin Sinclair** University of Nottingham
1600-1630	**Refreshment break and poster session**
1630-1645	Introduction to Tom Fleming: Introduction given by **Professor Keith Jones,** University of Southampton
1645-1715	**Professor Tom P Fleming**
1715-1845	Reception to celebrate Tom's retirement
1900-2200	Dinner

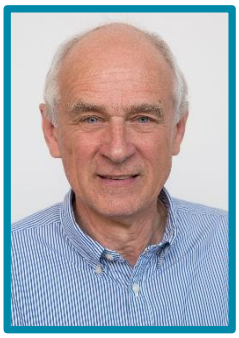
Invited Speaker Biographies

Professor Kate Hardy Imperial College London

Kate Hardy is Professor of Reproductive Biology in the Institute of Reproductive and Developmental Biology at Imperial College London, UK. She studied Natural Sciences at the University of Cambridge before working as a Research Officer with David Whittingham at the MRC Experimental Embryology and Teratology Unit in Carshalton, where she worked on parthenogenesis, embryo metabolism and development of mouse models of genetic disease, leading to her involvement in the new field of preimplantation genetic diagnosis (PGD). These interests continued following her move to the Royal Postgraduate Medical School, which later became part of Imperial College, based at Hammersmith Hospital in London where she undertook her PhD with Alan Handyside. She was involved in the first successful clinical PGD cases led by Robert Winston, and continued working in the area of human embryo metabolism (in collaboration with Henry Leese), as well as developing an interest in intercellular adhesion in the human preimplantation embryo. During her studies, it became apparent that healthy embryo development is dependent on healthy gametes, leading to her increasing interest in oocyte and follicle development in the ovary. Her current research, in collaboration with Stephen Franks, focuses on the cell biology of follicle development, specifically regulation of the activation of follicle growth and early preantral development.

Professor Henry Leese Hull York Medical School

Henry Leese is an Emeritus Professor of Biology in the Hull York Medical School. He graduated in Physiological Chemistry from Reading University and his PhD (Imperial College) was on the effect of diabetes on glucose transport and metabolism by the small intestine, an area he pursued during his first post doc position, at York University. After a sabbatical at the Swiss Federal Institute of Technology in Zurich studying the enzyme lactase, he returned to York and changed research direction to examine secretion by the mammalian female reproductive tract. Following a further sabbatical, in John Biggers' laboratory (Harvard), he began work on metabolism in preimplantation mammalian embryos. He is an FRCOG (*ad eundem*) and has Honorary Fellowships from the ACE, BFS and SRF, who awarded him the Marshall Medal in 2010. He was a member of the HFEA from 1998-2002 and is now on their Horizon Scanning Panel. He was founding Editor-in-Chief of *Human Fertility* and is currently BFS President. He gave the BFS Steptoe Lecture in 2016 and was awarded Honorary Membership of ESHRE the same year. He has supervised or co-supervised 30 PhD students and teaches human nutrition and metabolism and reproductive biology to undergraduates and postgraduates in biology and medicine.

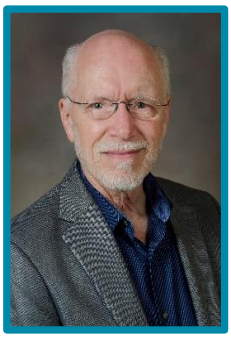


Professor Bernd Fischer Martin-Luther-Universität Halle-Wittenberg, Germany

Bernd Fischer is Emeritus Professor in the Department of Anatomy and Cell Biology at the Martin Luther University Halle-Wittenberg in Halle, Germany. He studied Agricultural Science (major Animal Science) at the University of Bonn and finished his Dr. agr. (PhD) in 1977 (supervisor Heiner Sommer). At the RWTH Aachen University he studied Human Medicine where he undertook his Dr. med. (MD) (1986) with Henning M. Beier. One year later he submitted the Habilitation thesis and was awarded with the Venia legendi for anatomy and reproductive biology. He did his postdoctoral training at the ARC Institute of Animal Physiology in Cambridge (UK) with Cyril E. Adams (1977-1978). As visiting professor he worked in the Department of Veterinary Science, University of Wisconsin, Madison (USA), in collaboration with Barry D. Bavister (1990-1991) and from 2001-2002 with Peter Kaye and Marie Pantaleon at the Department of Physiology and Pharmacology, School of Biomedical Sciences at the University of Queensland (Australia). He was Assistant and Associate Professor and Lecturer in the Department of Anatomy and Reproductive Biology at Aachen University Faculty of Medicine. From 1993 until 2015 he worked as Full Professor, Chairman and Head of Department in the Department of Anatomy and Cell Biology at the Martin Luther University Faculty of Medicine in Halle. He has published more than 130 peer-reviewed articles on preimplantation embryo development and gastrulation in mammals, on major metabolic pathways in preimplantation embryos (rabbit, mouse) and (human) stem cells, on prenatal programming of adult metabolic diseases by maternal metabolic disorders (diabetes type 1) and on disruption of embryo development and pregnancy by environmental endocrine disruptors.

Professor Alireza Fazeli University of Sheffield

Alireza Fazeli has the chair of Reproductive and Developmental Medicine at Sheffield University (United Kingdom) and the chair of Translational Medicine and Functional Genomics in Tartu University (Estonia). He has been the chair (Gemini) and the vice chair (Epiconcept) of two EU wide COST Action programs. Currently he is the vice chair and grant holder of a COST Action program (CellFit). His main research interests are in understanding intercellular communication, Epigenetics and Innate immunity. He has published over 250 scientific communications, which 90 are scientific papers appeared in multidisciplinary peer reviewed journals. In addition to professional scientific media, he has sought to disseminate his research in the public press and in different forms of media. From art objects exhibited in public art galleries to video clips on YouTube. His goal is to make science understandable for all, in particular the younger generations so as to inspire and educate them to pursue scientific careers. He hopes that his research make a difference in day to day lives of ordinary people.



Professor Kent Thornburg The Moore Institute, Portland, USA

M. Lowell Edwards Chair
Professor of Cardiovascular Medicine
Director, Center for Developmental Health, Knight Cardiovascular Institute
Director, Bob and Charlee Moore Institute for Nutrition & Wellness

Kent L. Thornburg, PhD, is the M. Lowell Edwards Chair of Cardiovascular Research, Professor of Medicine in the Knight Cardiovascular Institute at the Oregon Health & Science University. He holds joint professorships in the Departments of Physiology &

Professor Kent Thornburg continued

Pharmacology, Medical Informatics and Clinical Epidemiology and Obstetrics & Gynecology. He directs the Center for Developmental Health in the Knight Cardiovascular Institute and the OHSU Bob and Charlee Moore Institute for Nutrition & Wellness. He studies how women adapt to pregnancy and the roles of maternal diet and body composition in regulating fetal growth and lifelong health. He collaborates with scientists in England, New Zealand, Switzerland, Finland, Australia and India. He oversees clinical studies in rural Oregon and Alaska. Kent Thornburg serves regularly on advisory panels at the National Institutes of Health, the American Heart Association and the Children's Heart Foundation and serves on the medical advisory board of the Preeclampsia Foundation. He is director of research training for the Knight Cardiovascular Institute and holds grants from the NIH. He recently co-chaired the task force to determine the 10-year vision of the developmental origins of health and disease for the National Institute of Child Health and Human Development.

Professor Magdalena Zernicka-Goetz University of Cambridge

Magdalena is a Professor of Mammalian Development and Stem Cell Biology at the University of Cambridge. Her passion lies in understanding how cells decided their fate for the very first time and embryo builds its architecture.

Professor Daniel Brison University of Manchester

Professor of Clinical Embryology and Stem Cell biology; Scientific Director of the Department of Reproductive Medicine, Co-Director NW Embryonic Stem Cell Centre Department of Reproductive Medicine, St Mary's Hospital, Central Manchester and Manchester University Hospitals NHS Foundation Trust

Professor Daniel Brison is a Consultant Embryologist at St Mary's Hospital, Manchester and Person Responsible to the HFEA for licences in embryo research and embryonic stem cells. He is a member of the HFEA's Scientific and Clinical Advances Advisory Committee, the UK Association of Clinical Embryologists Scientific Advisory Committee, Clinical lead for the UK national MSc in Reproductive Sciences and an examiner for the Royal College of Pathologists. His clinical and research interests include: improving the effectiveness and safety of clinical assisted reproductive technologies (ART), the characterization of early human development at the molecular level, the regulation of pluripotency in embryos and embryonic stem cells and the derivation and use of clinical grade embryonic stem cells for the treatment of disease, and the impact of environmental factors and ART on embryonic and child health.

Dr Adam Watkins Aston University

Dr Watkins conducted his Ph.D within the laboratory of Professor Tom Fleming at the University of Southampton. Here, his research focused on investigating the impact of mouse embryo culture and environmental conditions on long-term adult health. During his PhD, Dr Watkins was the first to show that embryo culture and transfer procedures resulted in the development of hypertension and metabolic disorders in adult offspring. During his postdoctoral research, also with Professor Fleming, Dr Watkins assessed the impact of maternal low protein diet (LPD) given exclusively during pre-implantation development (3.5 days) in mice. Here, Dr Watkins observed that maternal preimplantation LPD increased offspring growth and adiposity, induced adult hypertension and vascular dysfunction and altered behavioural characteristics.
In 2011 Dr Watkins was awarded a University of Nottingham Advanced Research Fellowship to investigate the impact of paternal nutrition on sperm quality and adult offspring cardiovascular and metabolic health. Under this 2-year fellowship he demonstrated that LPD fed to male mice prior to conception induced genome wide hypomethylation in sperm, offspring hypotension, vascular dysfunction, glucose intolerance and elevated adiposity. Data from these studies identified potentials roles for both sperm- and seminal fluid-specific mechanisms through which paternal diet can affect offspring health and development.

In April 2014, Dr Watkins was awarded an Aston Research Centre for Healthy Ageing Research Fellow within Aston University to continue and develop his investigations into the impact of parental nutrition on offspring health. His current research is examining sperm- and seminal-fluid specific impacts on fetal growth, bone development and adult offspring cardiovascular and metabolic health.

Professor Kevin Sinclair University of Nottingham

Professor Sinclair's research interests are on the effects of assisted reproduction and parental nutrition on metabolic programming during early mammalian development, where epigenetic outcomes are determined in embryonic cells and tissues, and long-term developmental consequences assessed in offspring. First to discover that developmental anomalies following mammalian embryo culture were due to errors in genomic imprinting (Nature Genetics, 27: 153-154). Similar phenomena have since been reported in human IVF pregnancies. First to demonstrate that reductions in folate and vitamin B12 in the diets of intending mothers (rat and sheep) lead to epigenetic modifications to DNA methylation and adult offspring with increased body fat and blood pressure, altered immune function and insulin resistance (showcased to the NICHD Advisory Council in Washington DC in January 2007; PNAS, 104: 19351-19356). First to report comprehensive assessments of metabolic, cardiovascular and musculoskeletal health in aged offspring cloned by somatic-cell nuclear transfer (Nature Communications, 7: 12359-12369).

Invited Speaker Abstracts

Regulation of early ovarian follicle development

Professor Kate Hardy Institute of Reproductive and Developmental Biology, Imperial College London

In the ovary, the majority of follicles are at the primordial (resting) stage of development. Throughout reproductive life a steady trickle of follicles start growing, a process involving oocyte growth and increased proliferation of the enveloping granulosa cells. However, the mechanisms regulating this process remain unclear. Understanding these mechanisms is important because perturbations in the rate at which follicles initiate growth have a significant bearing on fertility, the age of the menopause and disorders of ovulation in women. Studies of follicle development in mice lacking specific genes, and in ovaries or pieces of ovary cultured under different conditions in vitro, suggest that early follicle development is mainly regulated by local growth factor signals, particularly by members of the Transforming Growth Factor (TGF)-beta superfamily. While a number of growth factors have been implicated in early follicle development, the source and identity of those that signal a specific follicle to start growing remain unresolved. In addition, it is not clear which cell type receives and responds to the signal; the oocyte or the granulosa cells. In this lecture, I will describe our use of image analysis to provide insight into the nature and source of the signals that activate follicle growth, as well as to explore the cell biology of follicles as they start growing.

Keep Calm and Carry On

Professor Henry Leese Hull York Medical School

The basic pattern of metabolism in mammalian oocytes and early embryos was established in the 1960s and 1970s, largely in terms of the utilisation of nutrients present in culture media at the time; mainly, glucose, pyruvate and lactate and of the consumption of oxygen. The potential importance of endogenous fuels was also recognised, especially by Kane, but was largely ignored, only to be rediscovered quite recently. Much of our current knowledge has been inspired by the need to improve embryo culture and selection methods in assisted reproductive technologies (ARTs). This pattern has continued alongside some basic metabolic studies and the general recognition of the importance of metabolism in all aspects of biology. Much of the data in the present talk derives from an MRC-funded Co-operative on *The Development of the Early Human Embryo* from which emerged the '*quiet embryo hypothesis*'. Recent developments of the hypothesis will be considered which illustrate the *Golidlocks Principle* and the notion of *Lagom*.

Metabolic profiling in embryos

Bernd Fischer, Maria Schindler, S. Mareike Pendzialek, Jacqueline Gürke & Anne Navarrete Santos

Department of Anatomy and Cell Biology, Martin Luther University Halle-Wittenberg Faculty of Medicine, D - 06097 Halle (Saale), Germany

Since the spectacular birth of the first baby conceived *in vitro* in July 1978, many studies have been performed to better understand embryo *in vivo* and *in vitro* development. Right from the beginning, a strong focus was laid on embryo metabolism as a prerequisite for development. First studies focused on non-invasive methods to judge embryo metabolism and developmental competence. The embryological and clinical landmark of the early nineties was the DOHaD theorem. Strong experimental evidence for DOHaD is owed to Professor Tom Fleming. He showed that the environment in which oocytes and preimplantation embryos develop does influence development and long- term health. Maternal diets fed as short as during the peri-conceptional period affect blastocyst development and subsequent fetal and offspring growth and health.

We have employed a diabetic rabbit model to learn more about the embryo's ability for adaptation, compensation and metabolic plasticity. Children born to diabetic mothers are having higher risks for congenital malformations and health problems later in life. We could show that maternal metabolic diseases such as diabetes mellitus type I change developmental

conditions and affect embryo development right from conception. First embryonic lineage decisions during gastrulation are altered by maternal diabetes. Albeit most embryos survive, blastocyst metabolism is changed. Thus, the prize paid for survival may be the misprogramming of the embryonic metabolism with long-term health effects. Currently, we are analyzing intra-embryonic cellular interactions by analyzing cell lineage-specific metabolic changes induced in a diabetic pregnancy, changes which potentially may lead to metabolic diseases later in life.

When males become pregnant....

Alireza Fazeli[1,2], Fran Otero[3], Freddy Lättekivi[2], William V Holt[1]

[1]Academic Unit of Reproductive and Developmental Medicine, Sheffield University, Sheffield, United Kingdom
[2]Department of Pathophysiology, Tartu University, Tartu, Estonia
[3] Grupo en Biodiversidad y Conservación, IU-ECOAQUA, Universidad de Las Palmas de Gran Canaria, Crta. Taliarte s/n, 35214 Telde, Spain

The thrifty phenotype hypothesis states that reduced foetal growth is strongly associated with the occurrence of a number of chronic non-communicable diseases such as coronary heart disease, stroke, diabetes, and hypertension in later life and during adulthood. This increased susceptibility results from adaptations made by the foetus in an environment limited in its supply of nutrients. Since its conception, the thrifty phenotype hypothesis has always been tested exclusively in one of the genders, i.e. females. The evidence provided to prove the validity of this hypothesis has always been limited in experiments following mothers. Furthermore, the majority of anecdotal evidence as well as epidemiological studies conducted have hardly been able to differentiate between the effect of factors such as nutrition, stress, climate changes, etc. on the environment in the womb during the periconception period and its effect on the embryo or the final maturational stages of female gamete either in ovary or in the womb. In this presentation, we discuss the tremendous potential provided by studying pregnancy in seahorses to test the effect of factors such as good and low-quality nutrition during the periconception period in pregnant males and its consequences on the health and quality of offspring produced. In short, we hope this model can give an answer to the question: “If dads could become pregnant, would they do a better job and produce healthier babies?”

Placental Programming: Vulnerable trophoblast

Kent L. Thornburg, Amy Valent, Melinda Pierce, Kevin Kolahi
Center for Developmental Health, Knight Cardiovascular Institute, Oregon Health and Science University, Portland Oregon

The relationships between placental size and shape and disease risk in offspring remain mysterious. The offspring of mothers who were preeclamptic and had thick placentas, die of stroke. Short placentas predict Hodgkin’s lymphoma in offspring. A small surface area of the delivered placenta is associated with heart failure in short mothers. A thin placenta in short mothers is associated with sudden cardiac death in men. The only solid conclusion from these epidemiological associations is that the placenta is a marker of biological processes that influence fetal development and vulnerability for disease. However, the mechanisms that underlie associations of placental phenotype and late life disease risk are not known. We need to pose helpful questions about how placental growth and shape are regulated. What nutrient signals might influence vascularization and polarized shape? What are the roles of specific cell types in regulating placental function? Our laboratory has focused on the roles of the mature trophoblast in regulating placental transport and metabolic activity. Thus far, we have learned that the cytotrophoblast has many previously unknown functions, some of which were once thought to be specific to the syncytiotrophoblast. For example, unlike syncytiotrophoblast, the cytotrophoblast is able to esterify free fatty acids and store them in lipid droplets. The cytotrophoblast consumes more oxygen and makes more lactate than does the syncytiotrophoblast. We conclude that the cytotrophoblast which has been previously relegated to a progenitor cell role, is a candidate for regulating the metabolic activity of the placenta and may be the most vulnerable cell layer to metabolic disruption. Thus, it may be an important biological target for programming the fetus.

Building the mammalian embryo - how to achieve a perfect partnership?
Professor Magdalena Zernicka-Goetz University of Cambridge

Mammalian embryogenesis requires intricate interactions between embryonic and extra-embryonic tissues to orchestrate and coordinate morphogenesis with changes in developmental potential. In both mouse and human embryos the first most dramatic series of morphogenetic transformations in embryo architecture and potency is initiated during embryo implantation into the body of the mother. Despite its major importance, an understanding of embryo remodeling during the implantation stage has been lacking, due to the embryo's inaccessibility within the mother. Motivated to understand this process, we have established a system permitting mouse and human embryogenesis beyond implantation in vitro. This allowed us to reveal steps of architectural remodelling and the importance of embryonic and extra-embryonic partnership in this process. Building upon this knowledge, we have attempted to mimic successive steps of this partnership with stem cells in vitro. To achieve this, we have combined mouse embryonic stem cells (ESCs) and extra-embryonic trophoblast stem cells (TSCs) in a 3D scaffold of extra-cellular matrix (ECM) that allowed us to generate structures whose morphogenesis is remarkably similar to natural embryos. By using genetically-modified stem cells and specific inhibitors, we show that embryogenesis of ESC- and TSC-derived embryos, ETS-embryos, depends on crosstalk involving Nodal signalling. When ETS-embryos develop further, they spontaneously initiate expression of mesoderm and primordial germ cell markers asymmetrically on the embryonic - extra-embryonic border, in response to Wnt and BMP signalling. This study demonstrates that enabling crosstalk between embryonic and extra-embryonic stem cells in a 3D ECM scaffold is sufficient to trigger self-organization recapitulating spatio-temporal events and leading to faithful reconstruction of embryo architecture and patterning. This stem cell model of mammalian embryogenesis, in combination with genetic manipulations, might provide a powerful platform to dissect physical and molecular mechanisms that mediate natural embryogenesis.

Is spending the first 5 days of life in a test tube good for your health?
Professor Daniel Brison University of Manchester

Clinical Assisted Reproduction Technology (ART) is now considered routine treatment with an estimated 6 million babies born globally since 1978. However, the pace of scientific and technological advances means that ART practitioners now have access to an increasing array of new and invasive technologies. In parallel with this, wider scientific and medical advances mean that we are increasingly aware of the potential impact of ART on embryonic development, gene expression, epigenetics, and the long-term health of ART children according to the Developmental Origins of Health and Disease (DOHaD). I will describe our research on the impact of ART on the transcriptome of human preimplantation embryos and cells, and on the birthweight and early growth of children arising from ART treatment. This work is funded by the UK MRC and the EU FP7 Health programme as part of the *EpiHealth and EpiHeathNet* consortia.

Early embryo environment and long-term offspring health
Dr Adam Watkins Aston University

Studies using human populations and animal species have shown that adult cardiovascular and metabolic disease risk is closely associated with poor environmental conditions experiences during the earliest stages of life. While the association between maternal diet and offspring adult health has been studied in detail, our understanding of the impact of paternal diet on offspring health remains largely neglected.

Using the well characterised rodent low protein diet (LPD; 9 % protein) model, we observe that offspring from LPD fed male mice have significantly increased weight at birth and adult body fat levels, impaired glucose tolerance and cardiovascular

dysfunction (indicative of obesity, type 2 diabetes and heart disease) when compared to offspring from control, normal protein diet (NPD: 18% protein) diet fed male mice.

Our latest studies have begun to define the underlying mechanisms linking paternal diet with perturbed offspring development and ultimately adult ill-health. During fetal development, we observe significant changes in embryonic metabolic status, fetal growth and skeletal formation and placental development and transport capacity. Our analysis of paternal and maternal tissues reveal genome wide paternal sperm DNA hypomethylation, altered testicular expression of epigenetic regulators and reduced maternal uterine signalling responses. These data indicate paternal diet may affect offspring development via sperm genomic and seminal fluid specific mechanisms. Our latest studies are aimed at defining the relative sperm and seminal fluid specific programming effects on offspring health and their persistence across generations.

One-carbon metabolism: linking nutritional biochemistry to epigenetic programming of long-term development

Professor Kevin Sinclair University of Nottingham

One-carbon (1C) metabolism consists of an integrated series of metabolic pathways that include the folate cycle and methionine remethylation and trans-sulfuration pathways. Most, but not all, 1C metabolic enzymes are expressed in somatic cells of the ovary, mammalian oocytes and in preimplantation embryos with subtle differences in expression existing between species. The metabolic implications of this, with regard to the provision of methyl donors, are not fully understood but mathematical models developed in house predict consequences for intra-cellular trans-methylation. These predicted effects are currently being tested experimentally both with ovarian somatic cells and zygotes cultured in vitro. However, we demonstrated previously in sheep that physiologically relevant reductions in the dietary supply of vitamin B_{12}, folate and this imethionine around the time of conception can epigenetically modify DNA in their progeny and lead to sex-biased insulin resistant and hypertensive offspring. Epigenetic alterations to DNA methylation in genes involved in key pathways associated with insulin signalling and endoplasmic reticulum stress have also been confirmed in adult offspring. Furthermore, we've observed similar sex-biased effects in offspring of rats fed folate, choline and methionine deficient diets. Focus has now turned to consider the contribution of polymorphic variances in genes encoding 1C enzymes, where initial studies have reverted back to the outbred sheep as a model species. Preliminary findings from these investigations will be presented.

Current research supported by BBSRC-IPA (BB/K017810/1) with AHDB, HCC and AgriSearch.

Poster List

P1	**Foetal size and sex influence placental and endometrial mRNA expression of angiogenesis related genes throughout gestation in the pig** **Stenhouse, Claire;** Hogg, Charis; Ashworth, Cheryl J *The Roslin Institute, University of Edinburgh, Midlothian, UK*
P2	**Radio frequency electromagnetic radiation from cell phone causes defective testicular function in male wistar rats** **Oyewopo, Adeoye** *University of Ilorin, Nigeria*
P3	**Effects of maternal high-fat diet (HFD) on cell populations in the cortex and hippocampus of the adult offspring mouse brain** **Ojeda Pedraza, Diego[1,2]**; Jayne-Coupe, Katherine[3]; Hutton, Oliver[2]; Fleming, Tom P [3]; Eckert, Judith[2]; Willaime-Morawek, Sandrine[2] *[1]University of Southampton, [2]Faculty of Medicine, University of Southampton, [3]Centre for Biological Sciences, University of Southampton, UK*
P4	**The possible beneficial effects of Naringenin against Highly Active Antiretroviral Induced Testicular Toxicity-Preliminary results** **Adana, Misturah Y** *Morphology and Andrology Group, Discipline of Clinical Anatomy, College of Health Sciences, University of KwaZulu-Natal, South Africa*
P5	**Metabolomics of porcine follicular fluid reveal the potential role of linoleic acid and erucic acid in oocyte maturation** **Jarrett, Selene[1]**; Gill, Andrew C[1]; Ferguson, Elizabeth M[2]; Ashworth, Cheryl J[1] *[1]The Roslin Institute and R(D)SVS, University of Edinburgh, [2]Aberdeen Maternity Hospital, UK*
P6	**Placental junctional zone Igf2 deficiency impairs maternal metabolic adjustments and blood volume expansion during pregnancy, with consequences for fetal growth in mice** **Yong, Hannah EJ[1]**; López-Tello, Jorge[1]; Sandovici, Ionel[2,3]; Constancia, Miguel[2,3]; Sferruzzi-Perri, Amanda N[1] *[1]Centre for Trophoblast Research, Department of Physiology, Development and Neuroscience, University of Cambridge; [2]Metabolic Research Laboratories, and MRC Metabolic Diseases Unit, Wellcome Trust-Medical Research Council Institute of Metabolic Science, University of Cambridge; [3]Department of Obstetrics and Gynaecology, University of Cambridge, UK*
P7	**Effect of low maternal protein diet in the reproductive system of adult bulls** **Ruiz-Diaz, Maria Dolores[1]**; Mongan, Nigel[1]; Copping, Katrina[2]; Alibhai, Aziza[1]; Keane, Matthew[1]; Purvis, Imogen[1]; Perry, Viv E A[1]; Rutland, Catrin S[1] *[1]School of Veterinary Medicine and Science, University of Nottingham, UK [2]Robinson Research Institute, University of Adelaide, Australia*
P8	**Regulation of endocytosis in embryos: Isoleucine depletion during mouse in vitro preimplantation development activates compensatory endocytosis in the trophectoderm** **Caetano, Laura[1]**; Sheth, Bhav[1]; Smyth, Neil[1]; Houghton, Franchesca[2]; Eckert, Judith[1]; Fleming, Tom P[1] *[1]Biological Sciences, University of Southampton, [2]Centre for Human Development, Stem Cells and Regeneration, Faculty of Medicine, University of Southampton, UK*
P9	**How safe is ART? Effect of in vitro fertilisation (IVF) and prolonged embryo culture on mouse development and postnatal health** **Aljahdali, Anan[1]**; Khalif, Ili[2]; Sheth, Bhav[1]; Velazquez, Miguel[3]; Wallen, Katrine[1]; Eckert, Judith[1]; Osmond, Clive[1]; Smyth, Neil[1]; Fleming, Tom P[1] *[1]University of Southampton UK, [2]University of Malaya, Malaysia, [3]Newcastle University, UK*

P10	**The effect of transforming growth factor (TGF)-β1 on metalloproteinase and tissue inhibitors of metalloproteinase in equine endometrial cells** Szostek-Mioduchowska, Anna[1]; Pacewicz, Joanna[2]; Okuda, Kiyoshi[3]; **Skarzynski, Dariusz Jan[1]** *[1]Department of Reproductive Immunology and Pathology, Institute of Animal Reproduction and Food Research of PAS, Olsztyn, Poland; [2]Department of Reproductive Immunology and Pathology, Institute of Animal Reproduction and Food Research, Olsztyn, Poland; [3]Obihiro University of Agriculture and Veterinary Medicine, Obihrio, Japan*
P11	**The consequences of maternal protein restriction around conception on mouse fetal brain development with a legacy for adult memory** **Gould, Joanna[1]**; Pearson-Farr, Jennifer[2]; Fleming, Tom P[3]; Willaime-Morawek, Sandrine[1] *[1]Clinical Neurosciences and Psychiatry, Faculty of Medicine, University of Southampton, Southampton, [2]Centre for Human Development, Stem Cells and Regeneration, University of Southampton, [3]Biological Sciences, University of Southampton, UK*
P12	**Why is fatty acid oxidation by mitochondria important in mouse oocytes?** **Bradley, Josephine**; Borri, Paola; Swann, Karl *Cardiff University, UK*
P13	**The effect of supplementation with folic acid in non-pregnant and pregnant mice on the ovarian morphology and embryo development** **Penailillo Escarate, Reyna Stephanie[1]**; Eckert, Judith[2]; Burdge, Graham[2]; Fleming, Tom P[1]; Lillycrop, Karen[1] *[1]Developmental Biology, University of Southampton, UK, [2]Academic Unit of Human Health and Development, University of Southampton, UK*
P14	**Weight and waist circumference of IVF children at the age of 9 years still affected by embryo culture medium** Zandstra, Heleen[1]; Feron, Frans[2]; Smits, Luc[2]; Brentjens, Linda[1]; Spauwen, Brenda[1]; van Golde, Ron[1]; Evers, Johannes[1]; Dumoulin, John[1]; **van Montfoort, Aafke[1]** *[1]Maastricht University Medical Center, [2]Maastricht University, The Netherlands*
P15	**Characterisation of genotypes associated with early pregnancy loss in the mare** **Smart, Harriette[1]; Rose, Belinda V[1]**; Hampshire, Daniel[1]; Ghosh, Shamila[2]; Raudsepp, Terje[2]; de Mestre, Amanda M[1] *[1]The Royal Veterinary College, Department of Comparative Biomedical Sciences, University of London, UK, [2]Department of Veterinary Integrative Biosciences, Texas A&M University, College Station, TX 77843, USA*
P16	**Optimising the culture conditions of mouse placental endocrine cells for downstream secretome analysis** **Napso, Tina** *Centre for Trophoblast Research (CTR), Department of Physiology, Development and Neuroscience, University of Cambridge, UK*
P17	**Over-expression of insulin-like growth factor-2 in the placental endocrine zone affects the metabolic profile of pregnant mice** **López-Tello, Jorge[1]**; Yong, Hannah EJ[1]; Grant, Imogen[1]; Boyland, Rebecca[1]; Sandovici, Ionel[2,3]; Constancia, Miguel[2,3]; Sferruzzi-Perri, Amanda N[1] *[1]Centre for Trophoblast Research, Department of Physiology, Development and Neuroscience, University of Cambridge; [2]Metabolic Research Laboratories and MRC Metabolic Diseases Unit, Wellcome Trust-Medical Research Council Institute of Metabolic Science, University of Cambridge; [3]Department of Obstetrics and Gynaecology, University of Cambridge, UK*
P18	**Genetics of one-carbon metabolism: Explaining inter-individual and ethnic variation in epigenetic responses to peri-conceptional diet** **Brassington, Amey H[1]**; Xu, Juan[1]; Matika, Oswald[2]; Kwong, Wing Yee[1]; Wragg, David[2]; Kendall, Nigel R[1]; Talbot, Richard[2]; Barrett, David A[1]; Archibald, Alan L[2]; Emes, Richard D[1]; Sinclair, Kevin D[1] *[1]University of Nottingham, Sutton Bonington, Leicestershire, UK, [2]Roslin Institute, University of Edinburgh, Easter Bush, Edinburgh, UK*
P19	**Mouse embryonic stem cell lines as models for periconceptional developmental programming** **Khurana, Pooja[1]**; Cox, Andy[2]; Fleming, Tom P[1]; Smyth, Neil[1] *[1]Centre for Biological Sciences, University of Southampton, [2]Department of Physiology, Development and Neuroscience, University of Cambridge, UK*
P20	**The influence of chemokines and immune cells on the function of porcine corpus luteum** **Andronowska, Aneta**; Witek, Krzysztof; Złotkowska, Aleksandra *Institute of Animal Reproduction and Food Research Polish Academy of Sciences, Olsztyn, Poland*

Poster Presentation Abstracts

P1

Foetal size and sex influence placental and endometrial mRNA expression of angiogenesis related genes throughout gestation in the pig

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Introduction: Appropriate prenatal growth and development is essential for post-natal survival and offspring growth. Inadequate foetal growth cannot be remedied post-natally which has severe consequences for neonatal and adult development. It is hypothesised that growth restriction occurs due to inadequate placental vascularisation.

Methods: Placental and endometrial samples associated with small and normal-sized, male and female Large White X Landrace foetuses were obtained at gestational day (GD) 18, 30, 45, 60 and 90 (n=5 or 6 litters/GD). The mRNA expression of angiogenesis related genes (uteroferrin (ACP5), platelet endothelial cell adhesion molecule (CD31), hypoxia-inducible factor (HIF1A), heparanase (HPSE), prostaglandin F2α receptor (PGFTR), secreted phosphoprotein-1 (SPP1) and vascular endothelial growth factor A (VEGFA)) was quantified by qPCR.

Results: Temporal changes in the expression of all candidates in both tissues were observed. Decreased expression of CD31 and SPP1 (GD60), HPSE and VEGFA (GD90), alongside increased HIF1A (GD45) expression were found in endometrial samples supplying small foetuses compared to their normal-sized littermates. ACP5 expression was increased (GD60), and CD31 (GD45 and 60) and HIF1A (GD90) were decreased in placentas supplying small foetuses compared to their normal-sized littermates.

Decreased endometrial expression of CD31, PTGFR and VEGFA in samples associated with male foetuses compared to female littermates occurred at GD30. At GD60, the observed differences were reversed, with increased endometrial expression of ACP5, CD31, SPP1 and VEGFA in samples associated with male foetuses compared to female littermates. Placental ACP5 expression was decreased at GD90 in samples associated with male foetuses compared to female littermates.

Discussion: The results of this study indicate that foetal size and sex influence the expression of angiogenesis-related genes in the porcine placenta and endometrium. These alterations are currently under investigation at a protein level to improve our understanding of how foetal size and sex influence angiogenesis at the feto-maternal interface.

Funding: Edinburgh University

P2

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

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

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

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

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

P3

Effects of maternal high-fat diet (HFD) on cell populations in the cortex and hippocampus of the adult offspring mouse brain

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Introduction: Maternal consumption of high-fat diet during gestation may be a risk factor for physiological and behavioural dysfunction in offspring. We test the hypothesis in mice that maternal HFD during pregnancy changes the structure and cellular organisation of the cortex and hippocampus regions of the adult offspring brain,

Methods: Female mice were fed different diets from conception: normal fat diet (NFD), HFD throughout gestation and lactation (HFD) or embryonic HFD (Emb-HFD) comprising HFD for 3.5 days and NFD thereafter. After weaning, all offspring were maintained on NFD. 6 male and 6 female brains were collected per group and analysed.

Results and Discussion: The Emb-HFD group presented an increase an increase in cell density (layer2/3 $p<0.05$; layer5 $p<0.05$) in $GFAP^+$ cell density (layer2/3 $p<0.05$; layer4 $p<0.01$; layer5 $p<0.05$) and $Iba1^+$ cell density (layer4 $p<0.05$, layer5 $p<0.01$) in the cortex. In the hippocampus, we saw an increase in $Iba1^+$ cell density (GCL<0.05).

In the HFD group, the cortex displayed a decrease in layer thickness (layer4 $p<0.0168$); an increase in cell density (layer1 $p<0.01$; layer2/3 $p<0.05$; layer5 $p=0.01$); an increase in $GFAP^+$ cell density (layer1 $p\leq0.0001$; layer2/3 $p<0.05$; layer4 $p<0.001$; layer5 $p\leq0.0001$; layer6 $p<0.01$) and an increase in $Iba1^+$ cell density (layer1 $p<0.001$; layer2/3 $p\leq0.0001$; layer4 $p\leq0.0001$; layer5 $p\leq0.0001$; layer6 $p<0.001$). Similarly, maternal HFD caused in the hippocampus a decrease in cell density

(SGZ: *p<0.01);* an increase in GFAP$^+$ cell density (SGZ *p<0.01)*; and an increase in Iba1$^+$ cell density (GCL *p<0.01*; SGZ *p<0.05*; Hilus *p<0.05*).

Our data show that maternal HFD increases astrocyte and microglia cell densities in both cortex and hippocampus. Taken together, these results suggest that maternal HFD consumption is critical for the development of the brain in the adult offspring. Further research will be important to confirm the inflammation status in these maternal HFD offspring brains.

P4

The possible beneficial effects of Naringenin against Highly Active Antiretroviral Induced Testicular Toxicity- Preliminary results

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Introduction: The era of Highly Active Antiretroviral Therapy (HAART) has witnessed a boost in the quality of life in people living with HIV/ AIDS giving them aspirations like non- infected persons even in terms of parenthood. The improved life span has however been shown to be accompanied by a surge in other health concerns including impaired fertility. This study aims to quantify the toxicity of one of the newer HAART regimen, ATRIPLA on the male reproductive capacity by assessing its effect on the testicular microanatomy using unbiased stereological methods.

Methods: Eighteen (18) male Sprague dawley rats were randomly divided into 6 groups viz- Group A: Control (Distilled water), B: HAART (Atripla). Group C: Naringenin, 40 mg/kg, Group D: Naringenin, 80 mg/kg, Group E: HAART + Naringenin, 40 mg/kg, Group F: HAART+ Naringenin, 80 mg/kg. The approval of Animal Research Ethical Committee of the University of KwaZulu-Natal, South Africa was obtained.
The animals were euthanized on the 28th day. The testes were excised and fixed in Bouin's fluid for histological analysis. The cauda epididymis from each animal were minced in 5 millilitres of normal saline and used seminal analysis. The histology of the excised testes was done using haematoxylin and eosin. The serum levels of reproductive hormones were determined. Level of apoptosis were assayed. Stereological methods were used determine the area and volumes occupied by the spermatogenic series and interstitium.

Results and Discussion: Results revealed that Atripla induced degeneration of seminiferous tubules and distortion of the normal architecture and orderly arrangement of the spermatogenic series which explains the abnormal semen parameters that was also observed.

Our results indicate that Atripla has deleterious effects on the testicular microanatomy of rats, which may impair fertility.

P5

Metabolomics of porcine follicular fluid reveal the potential role of linoleic acid and erucic acid in oocyte maturation

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Introduction: A diet high in fibre fed to gilts prior to ovulation enhances fertility, with more oocytes maturing to metaphase II during *in vitro* maturation (IVM) (Ashworth *et al*., 2008; *Proceedings of the Second Havemeyer Foundation Workshop on Embryonic and Fetal Nutrition* 27-29.). Additionally, blastocysts produced from the *in vitro* fertilisation (IVF)

of oocytes from high fibre fed gilts had more cells compared to blastocysts from control fed gilts (Ferguson *et al*., 2007, *Reproduction* **133**: 433-439). The current hypothesis is that the fatty acid composition of follicular fluid (FF) is altered by the diet which, in turn, confers reproductive benefits.

Methods: Fatty acids were extracted from the FF of 12 control-fed pigs and 12 high fibre-fed pigs with the Bligh and Dyer method. Within each feeding group, six pigs had positive IVF outcomes and six did not; a positive outcome was the formation of a blastocyst following IVF. Nine fatty acids (adrenic, arachadonic, arachidic, dihomo-γ-linolenic, docosapentaenoic, erucic, linoleic, palmitoleic and oleic) were measured in FF by tandem mass spectrometry and analysed by two-way ANOVA and t-tests.

Results and Discussion: The analysis revealed reduced concentrations of linoleic acid ($p \leq 0.05$) and increased concentrations of erucic acid ($p \leq 0.05$) in FF of high fibre-fed pigs compared to control-fed pigs. No fatty acids were statistically different between animals with successful IVF versus those with unsuccessful IVF. IVM media is being modified with the addition of different concentrations of linoleic acid to investigate their effects on cumulus expansion and oocyte maturity. Additionally, linoleic acid concentration in the maturation medium will be measured before and after IVM. The results of these *in vitro* experiments could influence the direction of future nutritional studies to refine pre-mating diets for improved fertility in female pigs and refine the composition of IVM media.

Funding: AHDB Pork and BBSRC.

P6

Placental junctional zone Igf2 deficiency impairs maternal metabolic adjustments and blood volume expansion during pregnancy, with consequences for fetal growth in mice

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Introduction: During pregnancy, the maternal ability to metabolically adapt and support fetal growth is facilitated by the placental endocrine output. Previous studies demonstrated that the paternally expressed imprinted gene, *Igf2*, drives placental endocrine cell formation in the murine junctional zone (JZ) and regulates fetal growth. However, the precise relationship between *Igf2*, placental endocrine function, maternal metabolism and fetal growth is unclear. The study aim was thus to examine the consequences of JZ *Igf2* deficiency on maternal metabolism and fetal growth.

Methods: Entire litters with endocrine JZ-specific *Igf2* deletion (Igf2del) were generated by time-mating female *TpbpaCre* mice with male *Igf2*-floxed mice. On pregnancy day 16, mice were either anaesthetised for blood collection or underwent a glucose or insulin tolerance test ($n \geq 3$ dams per genotype). Dams were schedule 1 killed and conceptus weights recorded. Lipid and haematocrit concentrations were determined in maternal blood. Age-matched dams from the reverse cross and non-pregnant mice served as controls. All animal experiments were performed with Home Office and local ethics approval.

Results and Discussion: Unlike that seen in pregnant reverse cross dams compared with non-pregnant controls, Igf2del dams failed to become glucose intolerant and insulin resistant, raise circulating triglycerides and expand their blood volume (as assessed from haematocrit) during pregnancy (one-way ANOVA, $p<0.05$). Igf2del dams also had lower circulating cholesterol and non-esterified fatty acids compared with reverse cross dams (Tukey's post-test, $p<0.05$). This observed failure to physiologically adapt during pregnancy was associated with smaller litter sizes and lighter fetuses in Igf2del dams (Student's *t* test, $p<0.01$). Therefore, *Igf2* expression in the JZ is important for adapting maternal physiology to support fetal growth and development during pregnancy. [This work was supported by funding from the Agency for Science, Technology and Research and the Royal Society.]

P7

Effect of low maternal protein diet in the reproductive system of adult bulls

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Introduction: Maturation of the bovine spermatozoa is achieved only after transit through the epididymis. This complex maturation process involves alteration of the membrane lipids, chromatin condensation, migration of the cytoplasmic droplet and changes in the acrosome. These modifications require the interaction of the spermatozoa with proteins synthesised and secreted by the epididymal epithelium. It is widely accepted that environmental perturbations during pregnancy may negatively affect the developing fetus. Numerous studies have focused on reproductive deficiencies produced in offspring following under- or over- nutrition *in utero*. Dietary protein is an important gestational dietary component in ruminants with its deficiency impacting the quality of the sperm later on in life.

Methods: In this large scale, farm based experiment we studied the impact of either, low or high dietary protein in nulliparous yearling heifers (n=360) during the peri conception (60 days before artificial insemination (AI) to 23 days post conception (dpc)) and/or first trimester of gestation (23dpc to 98dpc) upon the development of the epididymis in their 20 month old male offspring (n=40). Size, number and proportion of the epididymal tubules and blood vessels, as well as the amount of collagen surrounding the tubules were measured. In addition, the proteomics of the epididymal fluid were analysed.

Results and Discussion: This study provides an insight into the epididymal structural and proteomic alterations in male adult offspring consequent to differing *in utero* protein environments. The observed effects have the potential to reduce fertility via alterations to the sperm maturational process within the epididymis.

P8

Regulation of endocytosis in embryos: Isoleucine depletion during mouse in vitro preimplantation development activates compensatory endocytosis in the trophectoderm

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Introduction: We have shown mouse maternal low protein diet during the preimplantation period (Emb-LPD) causes chronic disease in adult offspring. Emb-LPD causes depletion of branched-chain amino acids (BCAA) in maternal uterine luminal fluid and insulin in serum, recognised by embryo nutrient-sensing mechanisms, leading to compensatory responses (e.g. increased endocytosis in the blastocyst trophectoderm, TE) to promote adaptation to poor nutrition. This study determined the role of individual BCAA and insulin depletion in stimulating endocytosis in TE using an *in vitro* embryo culture model.

Methods: 2-cell embryos were cultured to the blastocyst stage in KSOM medium supplemented with BCAA and insulin at normal (N, 100%) or with individual BCAA or insulin depleted by 50% (L-): low valine (L-VAL+N-INS), low isoleucine (L-ISO+N-INS), low leucine (L-LEU+N-INS), low insulin (NBCAA+L-INS) and a control group (N-BCAA+N-INS). Control medium was supplemented with serum insulin (1 ng/ml) and uterine luminal fluid amino acid concentrations found in well-fed mice. After culture, blastocyst fluorescent markers for lysosomes and endocytosed and processed BSA were analysed using confocal microscopy and VOLOCITY imaging software.

Results and Discussion: Blastocysts cultured in medium with depleted isoleucine (L-ISO+N-INS) but not other nutrients showed increased lysosome number and BSA degraded particles compared to the control group (50% and 60% respectively, P<0.05%). These data indicate an essential role for isoleucine specifically in embryo nutrient sensing around the time of conception with long-term consequences for health and disease risk. (Funding: Rosetrees Trust; University of Southampton).

P9

How safe is ART? Effect of in vitro fertilisation (IVF) and prolonged embryo culture on mouse development and postnatal health

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Since the advent of IVF (*in vitro* fertilisation), several million babies have been born worldwide. However, reports link *in vitro* techniques with adverse short and long-term health outcomes. Using a mouse model, we investigated the effect of IVF and duration of culture on blastocyst development and cell number and the postnatal health of offspring. Experimental groups (8-13 litters each): NM (natural mating, non-superovulated); IV-ET-2Cell (2-cell embryos derived *in vivo* from superovulated mothers (SOM) and immediately transferred (ET) to recipients; IV-ET-BL (blastocysts derived *in vivo* from SOM and immediate ET); IVF-ET-2cell (2-cell embryos generated by IVF from SOM, short culture and ET); IVF-ET-BL (blastocysts generated by IVF from SOM, long culture and ET). IVF blastocysts after prolonged culture developed slower and comprised reduced trophectoderm and ICM cell numbers compared with in vivo generated blastocysts (P<0.05; n= 50-87 per treatment). IV-ET-2Cell (n= 57), IV-ET-BL (n= 47), IVF-ET-2Cell (n= 75) and IVF-ET-BL (n= 42) groups compared with NM controls (n=80), showed increased body weight, increased SBP, impaired GTT and abnormal organ:body weight ratios in both genders (P<0.05), independent of litter size. SBP and Angiotensin Converting Enzyme (ACE) for IVF-ET-BL males was increased compared to IV-ET-BL males. SBP for IVF-ET-BL males was increased compared to IVF-ET-2Cell males. However, glucose concentration 2 hours after glucose injection and AUC (area under curve) in male IVF-ET-BL was reduced compared with IVF-ET-2Cell males. Serum insulin for IVF-ET-BL males was significantly reduced compared with IVF-ET-2Cell, but serum glucose and G:I ratio did not show any significant differences. In conclusion, reproductive treatments affect the development and potential of preimplantation embryos, influencing postnatal development and physiology compared with undisturbed reproduction. In particular, prolonged embryo culture, with normalised SO, IVF and ET, may adversely affect male offspring cardiovascular but improve the metabolic profile compared with short culture, However, female health is less sensitive.

P10

The effect of transforming growth factor (TGF)-β1 on metalloproteinase and tissue inhibitors of metalloproteinase in equine endometrial cells

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Transforming growth factor-β1 (TGF-β1) regulates cell growth, development, and tissue remodeling and wound healing. TGF-β1 affects the tissue remodeling process by increasing expression of extracellular matrix (ECM) proteins, tissue inhibitors of metalloproteinase (TIMPs) and decreasing synthesis and activity of metalloproteinase (MMPs). The primary function of MMP is the degradation and remodeling of ECM. In turn, TIMPs play an important role in establishing the *balance* of *ECM component synthesis* and *degradation.* TGF-β1 plays important roles in regulation of uterine function in

number of species. However, in mares concentration of TGF-β1 is up-regulated in equine endometrial fibrosis (*endometrosis*). The aim of study was to determine if TGF-β1 takes part in endometrial tissue remodeling by acting on MMPs and TIMPs.

Equine endometrial cultured fibroblasts (n=6) and epithelial (n=5) cells were stimulated with vehicle or TGF-β1 (5 ng/ml) for 24 or 48 h. Then, mRNA expressions of *MMP-1*, *-2*, *-3, -9* and *-13* and *TIMP-1*, *-2* were determined using Real-time PCR. The secretion level of MMPs was determined using ELISA.

TGF-β1 did not affect the secretion of MMP-2 and TIMP-2 and its mRNA transcription in fibroblasts (P>0.05). In turn, TGF-β1 in time depending manner increased secretion of MMP-1, -3, -9 and MMP-13 in fibroblasts (P<0.05). Additionally, TGF-β1 did not affect the secretion of MMP-2, -3 and MMP-13 (P>0.05), but increased the secretion of MMP-1, -9 and TIMP-1, -2 from epithelial cells (P<0.05).

In equine endometrial cells, the expression and secretion of MMP-1,-3,-9,-13 and metalloproteinase inhibitors is regulated by TGF-β1. Thus, the data suggest the participation of MMPs and their inhibitors originated in epithelial cells and fibroblasts in physiological and also pathological remodeling of the endometrial tissue.

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P11

The consequences of maternal protein restriction around conception on mouse fetal brain development with a legacy for adult memory

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Introduction: Maternal malnutrition during pregnancy is detrimental to fetal development and increases the risk of chronic diseases in later life including neurological consequences such as schizophrenia. Previously, such studies have shown brain development in late gestation and after birth to be affected, influencing structural, biochemical and pathway dynamics for motor and cognitive function. However, the importance of nutrition during embryogenesis for brain development is unknown. We have previously shown maternal low protein diet confined to the preimplantation period (Emb-LPD) in mice with normal nutrition thereafter and postnatally is sufficient to induce cardiometabolic and locomotory behavioural abnormalities in adult offspring.

Methods: Virgin female mice were fed from mating to term (1) normal protein diet (NPD), (2) low protein diet (LPD) or (3) embryonic LPD (Emb-LPD; LPD for 0-3.5 days (E3.5), NPD thereafter). Fetal brains were analysed at E12.5, E14.5 and E17.5, using flow cytometry, immunofluorescence and neurosphere culture assays for evaluation of neural stem cell (NSC) and differentiation dynamics. Behavioural tests for memory including novel object recognition and T- Maze were performed in adult offspring.

Results & Discussion: Emb-LPD and sustained LPD reduced NSC and progenitor cell numbers through suppressed proliferation rates (P<0.05) and increased apoptosis (LPD P<0.001) in both ganglionic eminences and cortex of fetal brain at E12.5, E14.5 and E17.5. Emb-LPD additionally caused rapid differentiation of remaining NSCs (P<0.01). Emb-LPD adult offspring exhibited a deficit in short-term and long-term memory (P<0.00001).

These data reveal for the first time that poor maternal nutrition around conception, even with good nutrition thereafter, is

sufficient to compromise fundamental cellular processes coordinating early brain development and differentiation, associated with adult offspring memory deficits. (Funding Rosetrees Trust; BBSRC; Faculty of Medicine, Southampton)

P12

Why is fatty acid oxidation by mitochondria important in mouse oocytes?

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Introduction: Mammalian oocytes generate ATP from mitochondrial oxidation of fatty acids (FAO) and pyruvate. Mouse oocytes store FAs as triglycerides in lipid droplets (LDs) (Bradley et al 2016; Dev 143: 2238-2247). Inhibition of mitochondrial FA transport using etomoxir has been found to inhibit oocyte maturation and embryo development (Dunning et al 2010; Biol Reprod 83: 909–918), suggesting an important role of FAO in development. The aim of this study is to determine why FAO and LDs are important for mouse oocytes and embryos.

Methods: ATP levels were assayed using chemiluminescence imaging of microinjected firefly luciferase in intact mouse oocytes *in vitro*, and autofluorescence was measured to assess the reduction state of mitochondrial-specific FAD^+. Dihydroethidium was used as a fluorogenic probe for production of reactive oxygen species (ROS) such as the superoxide anion. Etomoxir was used to inhibit FAO and cynano4-hydroxycinnamate (CHC) to inhibit pyruvate transport.

Results and Discussion: Etomoxir addition to oocytes resulted in a small decrease in ATP, followed by recovery and slight increase, suggesting compensatory metabolism. Recovery appeared to be due to pyruvate oxidation since there was no recovery of ATP levels when etomoxir was added in the presence of CHC. FAD^+ autofluorescence from oocytes and early embryos showed an unexpected decline in response to etomoxir, again suggesting increased pyruvate oxidation with FAO inhibition. Increased pyruvate oxidation may be detrimental to oocytes as we found ROS production was stimulated by etomoxir in a manner that was enhanced by higher pyruvate concentrations.

These data suggest FAO is not essential for ATP production in mouse oocytes since mitochondria can compensate for loss of FAO by taking up pyruvate, however compensatory pyruvate oxidation enhances ROS production. FAO may play of role in suppressing ROS generation in mouse oocytes and early embryos.

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P13

The effect of supplementation with folic acid in non-pregnant and pregnant mice on the ovarian morphology and embryo development

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Introduction: Women of reproductive age are recommended to take folic acid (FA) to prevent neural tube defects during pregnancy. There is some evidence that indicate that FA could improve fertility. However, further research is needed to identify the effect of FA supplementation on the ovary and embryo development. The aim of this study is to determine the impact of FA supplementation on ovarian morphology and the potential consequences for the embryo.

Materials and Methods: Adult (10w old) female C57BL/6 mice were fed for 4w standard (1 mg/Kg) or high (5 mg/Kg) amounts of FA. The animals were then either culled at diestrus or mated and then culled 3.5 days post coitum (dpc). Embryos were collected and frozen at -80C. One ovary per female was used for qRT-PCR and the other was fixed for

histological analysis.

Results and Discussion: In non-pregnant mice FA supplementation decreased ovarian FSH receptor, Brca1 and BMI1 expression. However, there were no significant differences in the number of follicles between dietary groups. Pregnant mice showed a 30% lower number of embryos at 3.5 dpc after supplementation with FA. These results suggest that FA supplementation could affect adversely follicular metabolism and embryo development.

P14

Weight and waist circumference of IVF children at the age of 9 years still affected by embryo culture medium

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Introduction: Previously, we have shown that after alternating assignment to embryo culture in either K-SICM (Cook, Brisbane,Australia) or G1™ Version3 (Vitrolife, Götenborg, Sweden), birthweight of the resulting IVF children was significantly higher in the Vitrolife group. This weight effect persisted during the first 2 years of life. The aim of the present study was to investigate growth and body composition of these singleton IVF children at the age of 9 years.

Methods: In this study, children from the abovementioned study were invited to attend our clinic for anthropometric measurements as a part of the MEDIUM-KIDS study. Of the 294 eligible children included in the original study, 136 children (75 Vitrolife and 61 Cook) participated in the current study. Two experienced clinicians performed the following measurements in a standardized way: height and weight of the child, 4-point skinfold thickness measurements in threefold and waist/hip circumference. The study was approved by the local Ethics Committee.

Results and Discussion: Baseline characteristics between the two groups were similar. Although height and height corrected for age and gender (SDS scores) were similar in both group, weight and weight SDS scores were higher in the Vitrolife group as compared to the Cook group (34.2kg ± 6.6 vs 32.1kg ±5.9 (P=0.06) and -0.51 vs 0.11 (P=0.02)). After correction for several potential confounding factors, the difference in weight attributable to culture medium was 1.7kg (*P*=0.047). Furthermore, waist circumference, waist/hip ratio, subscapular skinfold and truncal adiposity were significantly higher in the Vitrolife group. After correction for confounders the difference attributable to culture medium was 2.7mm (*P*=0.044) and 0.030 (*P*=0.015) for waist and waist/hip ratio and 0.15cm (*P*= 0.08) for truncal adiposity. The peripheral skinfolds biceps and triceps were similar.

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P15

Characterisation of genotypes associated with early pregnancy loss in the mare

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Introduction: Early pregnancy loss (EPL) affects 5-10% of equine pregnancies and is the largest contributor to nationwide reproductive wastage, of which 80% of cases are of unknown cause(s). Chromosomal abnormalities are likely to be a

significant contributor to EPL, especially given its increased rates in older mares. The exact role of genetic aberrations in EPL in horses is, however, unknown. We recently identified 17 novel (not previously described in the horse) copy number variation regions

(CNVR) in 12 failed early pregnancies. Our objectives were to compare (i) CNVs in healthy and failed early pregnancies and (ii) CNV over time, between healthy early and healthy term pregnancies.

Methods: Bioinformatic profiling of 17 novel CNV regions identified two regions of interest; CNVR Chr28:18833995-18846757 and CNVR ChrX:98506468-98543836, which encompassed genes relating to post replication repair and sister chromatid cohesion respectively. Real time qPCR reactions (two sites per CNVR) were performed to quantify the CNV in three groups of allantochorion samples; EPLs (n=16), healthy early (HE) (n=19) and healthy term (HT) (n=9) pregnancies. Relative genetic expression was determined using the 2-ΔΔCq method. Results were analysed using One Way ANOVAs in GraphPad Prism 7. Significance was set as $p < 0.05$.

Results and Discussion: There was no significant difference in average relative fluorescence between EPL and HE samples both within the genic and intergenic regions of the CNVR on chromosome X, nor in either of the regions of the CNVR on chromosome 28. There was also no significant difference in average relatively fluorescence for CNVR between HE and HT samples. For both of the CNVRs, there was significant variation in relative fluorescence in the placentae of both healthy and failed pregnancies. Further studies with larger group numbers and involving additional CNVR are needed to better understand the role of submicroscopic chromosomal changes in EPL.

P16

Optimising the culture conditions of mouse placental endocrine cells for downstream secretome analysis

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Introduction: During pregnancy, many physiological changes occur in the mother to enable fetal growth support. These include changes in the maternal cardiovascular and metabolic systems, and are signalled, in part, by changes in placental hormone production. However, complete identification of the proteins secreted by the placenta that mediate the changes in maternal physiology is lacking. This study aimed to establish a culturing method for placental endocrine cells from which secretory output could be collected and characterised.

Methods: The mouse placenta was used as the endocrine and transport functions are performed by discrete zones that can be physically separated. Mice were handled according to local ethical requirements. Whole placenta (WP) and isolated endocrine junctional zones (Jz) were obtained from schedule 1 killed dams on day 16 of pregnancy (maximal Jz size). WP and Jz trophoblast were then isolated by density-gradient centrifugation, seeded at 10^4cell/ml and cultured for up to 120h (n=6).

Results and Discussion: In both WP and Jz cultures, ~20% of the cells were no longer viable at 24h of culture, and a further 20% were lost by 48h. Cell proliferation was detected at 72h and 96h, probably reflecting fibroblast contamination and expansion. By 120h, the majority of WP and Jz cells were not viable. In Jz cultures, the concentration of protein in conditioned media was ~400µg/ml at both 24h and 48h, whereas at 72h, the concentration was ~240µg/ml (n=2). Protein content of the WP conditioned media was ~100µg/ml at both 24h and 48h (n=2). Density of endocrine versus transport trophoblast cells in the WP and Jz cultures are being investigated. Once the optimal culturing conditions have been established, mass spectrometry will be performed to define the secretome of placental endocrine cells.

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P17

Over-expression of insulin-like growth factor-2 in the placental endocrine zone affects the metabolic profile of pregnant mice

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Introduction: Insulin-like growth factor-2 (*Igf2*) is a growth promoting, paternally-expressed imprinted gene. It is highly expressed in feto-placental tissues and is required for normal conceptus growth and placental nutrient transport. However, less is known about the role of *Igf2* in placental endocrine function and putative adaptation of maternal metabolism to favour resource allocation. This is important as failure to appropriately adapt maternal metabolism may lead to abnormal birthweight and gestational diabetes. This study aimed to determine the effect of over-expressing *Igf2* in the placental endocrine junctional zone (Jz-*Igf2*OE) on maternal metabolism and feto-placental growth in mice.

Methods: Transgenic mice were crossed to produce entire litters with Jz-*Igf2*OE using the *TpbpaCre* line (activation of the normally silent maternal *Igf2* allele in the placental junctional zone via conditional deletion of the *H19DMR*). Procedures were performed abiding to the UK Home Office Animals Act 1986. On day 16 of pregnancy (term ~20 days), dams underwent a glucose tolerance test or were killed under terminal anaesthesia for blood collection. Following schedule 1 killing, conceptus weights were recorded, placentas taken for structural analysis and maternal tissues collected for molecular analyses. Data were compared to pregnancies with unaltered placental *Igf2* expression.

Results and Discussion: Circulating glucose, insulin and leptin concentrations were increased and free fatty acids reduced in Jz-*Igf2*OE dams. However, glucose tolerance and glucose-stimulated insulin production were not affected in Jz-*Igf2*OE dams. The abundance of insulin signalling and glucose transporter proteins was diminished in the adipose tissue and unchanged in the skeletal muscle of Jz-*Igf2*OE dams. Placental weight was increased and related to an expansion of the placental endocrine region with Jz-*Igf2*OE. Fetal weight was unchanged by Jz-*Igf2*OE. Therefore, *Igf2* is important for regulating placental endocrine zone formation and maternal glucose-insulin handling. Work is underway to further assess changes on day 19 of pregnancy.

P18

Genetics of one-carbon metabolism: Explaining inter-individual and ethnic variation in epigenetic responses to peri-conceptional diet

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Introduction: Dietary disturbances to one-carbon metabolism during the peri-conceptional period can lead to early miscarriage and compromise offspring health, in part due to epigenetic dysregulation of chromatin methylation in developmentally important genes. We hypothesise that inter-individual and ethnic variability in epigenetic gene regulation arises because of single-nucleotide polymorphisms (SNPs) within metabolic genes, epigenetic regulators and differentially-methylated target DNA sequences. We report on ongoing studies that seek to test this hypothesis in sheep.

Methods: Twenty unrelated Texel ewes were sequenced to a depth of 30X in two pools. Reads were mapped to the reference sheep genome assembly (Oar_v3.1), identified variants were used to construct an Illumina Infinium® iSelect® Custom Array (6,000 probes), capturing 140 one-carbon metabolism genes and 116 related epigenetic regulators. This array was used to SNP profile 270 Texel-sheep liver samples for which we also quantified 116 one-carbon and related metabolites by LC-MS/MS. SNP variants were also determined in 190 unrelated pedigree sheep from three traditional UK breeds (Suffolk, Bluefaced-Leicester and Swaledale).

Results and Discussion: Genetic quantitative trait association analyses (by GenABEL in R) on metabolite concentrations were adjusted for substrates, co-factors and allosteric regulators. Following Bonferroni correction, and re-estimation in ASReml, a prioritised list of 27 SNPs in 15 metabolic genes and 54 SNPs in 25 epigenetic-regulator genes was generated, consistent across covariate categories. Of these, four SNPs (upstream variants) in *PCMTD1* (protein methylation; allele frequencies (q) of 0.43) and three SNPs (two missense variants; q of 0.26 for each) in *RRP8* (a histone methyltransferase) were linked to tetrahydrofolate. K-means clustering and phylogenetic analyses indicated a high degree of breed divergence for our 256 genes; with the three UK breeds clustering separately from Texels. Future studies will confirm/extend prioritised SNPs in one-carbon deficient animals and undertake a prospective proof-of-concept pregnancy study with each breed.

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P19

Mouse embryonic stem cell lines as models for periconceptional developmental programming

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Introduction: Developmental Origins of Health and Disease (DOHaD) proposes maternal environment during pregnancy influences adult offspring disease risk. Programming may occur within the preimplantation embryo. We have shown maternal low protein diet (LPD) and advanced maternal age (AMA) programme the mouse embryo to altered postnatal growth and cardiometabolic dysfunction.

Materials and Methods: (i) mESCs from blastocysts from mothers fed LPD (9% casein) and normal protein diet (NPD, 18% casein); from AMA (7-8 months) and young (7-8 weeks) dams (ii) Cell lines were characterised for derivation efficiency, karyotype, gender. (iii) Normal lines were assessed for developmental programming mechanisms.

Results and Discussion: Both groups show similar mean percentage of embryos derived i.e., 62% from AMA % and 70% from control dams, similar to our *in-vivo* data. All mESC lines show similar derivation efficiency per embryo (45% AMA; 36% young) resulting in 28 AMA and 10 young mESC lines with a dominance of male gender lines 80% vs 90% respectively. AMA lines showed higher aneuploidy (28.5%) than young (16.6%). Lines are being analysed for proliferation rate, apoptosis and gene/protein expression. Early data shows AMA lines have reduced viability and increased cell death. LPD lines preserve cellular and epigenetic programming changes inherited from embryos. Metabolomics of LPD vs NPD lines (with Metabolon Inc) shows increase in glucose metabolism, fatty acid homeostasis and ascorbate utilization. LPDs exhibited significant increase in glucose 6-phosphate and fructose 6-phosphate with reduced downstream metabolites, implicating glycolytic enzyme expression such as phosphofructokinase may be altered, contributing to LPD programming. These changes were consistent with changes in carbon flow. These variations indicate maternal LPD induces heritable metabolic reorganisation as an early mechanism in developmental programming.

P20

The influence of chemokines and immune cells on the function of porcine corpus luteum

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The main function of the corpus luteum (CL) is secretion of progesterone (P4) to establish and maintain pregnancy. If pregnancy is does not occur, CL undergoes functional and structural luteolysis. Moreover, during CL regression, the number of immune cells significantly increases. There is growing evidence that chemokines produced by immune components may be directly or indirectly involved in the regulation of CL function. This study was designed to investigate the chemokine expression in porcine CL and in vitro effect of PGF and chemokines on luteal cells function.
CLs collected from mature crossbred gilts on days 10, 12, 14 of the estrous cycle/pregnancy were used to determine chemokines expression using qPCR and WB. Purified PBMCs and PMNs were analyzed for migration and activation after PGF and chemokine treatment. Isolated luteal cells from day 12 of the cycle were used to determine the effect of chemokines, PGF, PMNs and PBMCs on P4 production, proliferation and migration. Data were analyzed by two-way Anova followed by Tukey's post hoc test.

The increased expression of CXCL10, CXCL9 and CCL8 on day 12 and CXCL2, CXCL8, CCL2, CCL4, CCL5 on day 14 of the estrous cycle was found. Both activated PMNs and PBMCs inhibited P4 production. Significant decrease in P4 synthesis by mixed luteal cells was also observed after PGF and CCL2, CXCL2, CCL4, CCL8 treatment. CXCL8 had the strongest effect on PMNs and PBMCs migration. PGF did not affect either PBMCs proliferation or PBMCs and PMNs migration. All analyzed chemokines, except CCL5 and CXCL8, stimulate PBMCs migration. CXCL8 was the strongest chemoattractant for PMNs. PMNs, CCL8 and CXCL12 stimulate endothelial cells proliferation. CCL4, CXCL9 and CXCL12 affect tube-like structure formation. Summarizing, chemokines and immune cells affect luteal cell function by inhibiting progesterone production and modulation of angiogenesis in porcine CL. Supported by NSC grant 2012/05/B/NZ9/03330

Author Index-Submitted Abstracts

Notes