Contents

Welcome

Welcome to the Society for Reproduction and Fertility's (SRF) Annual Conference 2010 at Sutton Bonington Campus, University of Nottingham, UK. This meeting marks the Society's Diamond Jubilee and a very special symposium has been organised to celebrate this particular anniversary.

This meeting will as always be attended by members of the scientific community working within research and academic institutions across the world. At a time when the general area of Reproduction and Fertility is coming under pressure from all quarters, it is nice to be able to welcome you all to an event that celebrates the science.

For the 2010 conference we have an inspiring programme spanning many disciplines of reproductive biology. Social events are structured to promote networking and interaction between people working in the key disciplines of reproduction and fertility. These include the Drinks Reception followed by the SRF Quiz Night on Sunday and the Conference Dinner held on Monday night. This year's conference dinner will see the return of the "Ceilidh" which I hope you will ALL enjoy.

This year the recipient of the Society's Marshall Medal is Henry Leese who will be presented with this prestigious Award during the Conference Dinner. The Society's various other prizes (Post Doc Scientist, SRF Student Prize and Poster Prizes) will also be presented at the Conference Dinner.

The conference programme includes the traditional PLENARY LECTURES: the Distinguished Scientist (Steve Franks), SSR New Investigator (Wei Yan) and SRF New Investigator (Suzannah Williams), MULTIPLE SYMPOSIA *(Molecules of Reproduction, Reproduction and Development in exotic species, Animal models: how relevant are they? and the SRF 60th anniversary celebration).* Delegates are in for a real treat! The programme also includes a number of FREE ORAL COMMUNICATIONS in the general areas of *Female/Male Reproduction, Ovary/Uterus and Oocyte/Embryo* together with nearly 80 POSTER Presentations. The ever popular SRF STUDENT PRIZE and the POST DOCTORAL PRIZE sessions were chosen from the strongest field to date, also feature in the programme. All posters will be available for viewing throughout the conference and will be manned by the authors during the designated poster "sessions". A new feature at this year's conference will be "a poster prize chosen by the delegates". We hope delegates will enter into the spirit and participate wholeheartedly!

I would like to take this opportunity to thank the SRF Council and members of the programme committee for their hard work and advice in organising this conference.

It is a pleasure to welcome you to SRF 2010 in the beautiful setting of Sutton Bonington. Whether you are attending your 1st or 60th SRF conference, I hope you have a truly memorable time at SRF 2010.

Dr Robert Abayasekara
SRF Programme Secretary

General Information

Sutton Bonington and venue

The Sutton Bonington Campus is 10 miles south of the main University on the border of Nottinghamshire and Leicestershire. Its countryside location makes an ideal home for the School of Biosciences and the University's new School of Veterinary Medicine and Science. The School of Veterinary Medicine and Science was launched in 2006, in new purpose-built facilities - a landmark as the UK's first new Vet School since the 1950s.

The 100 acre campus is amply equipped with:

- state-of-the-art teaching and research facilities
- student residences
- a new sports centre
- essential amenities including a bank and bookshop

For further information about the venue please visit www.nottinghamconferences.co.uk

Car Parking

Parking is available on campus and it is free of charge. There are 250 spaces and each one is allocated on a first come, first served basis.

Accommodation and Breakfast

For anyone booked on a residential package, onsite accommodation will be provided during the conference.

Check-In and Check-Out

En-suite student accommodation is provided during this event in the Costock Hall of Sutton Bonington Campus. You can check in for accommodation and register your attendance at the conference in the Atrium of the Vet School Building. Accommodation will be available from 1500. If your bedroom is not ready for you on your arrival then a luggage store will be available.

Please check out of your room before 1000 on the day of departure. Please be aware that fines will be charged for any late checkouts.

Accommodation Facilities

Accommodation comprises of en-suite bedrooms. Guests are reminded that bedrooms are basic student accommodation.

Please note that all bedrooms are single occupancy only. The venue do not offer twin or double occupancy rooms. Unauthorised dual occupancy violates the University's Health and Safety Regulations. All of the buildings on site are non-smoking, including all bedrooms.

Breakfast will be served in the main restaurant.

Internet

Wireless Internet is available across the campus and in the foyer and common room of the accommodation block. Wired Internet is available in bedrooms.

Medical and Security Information

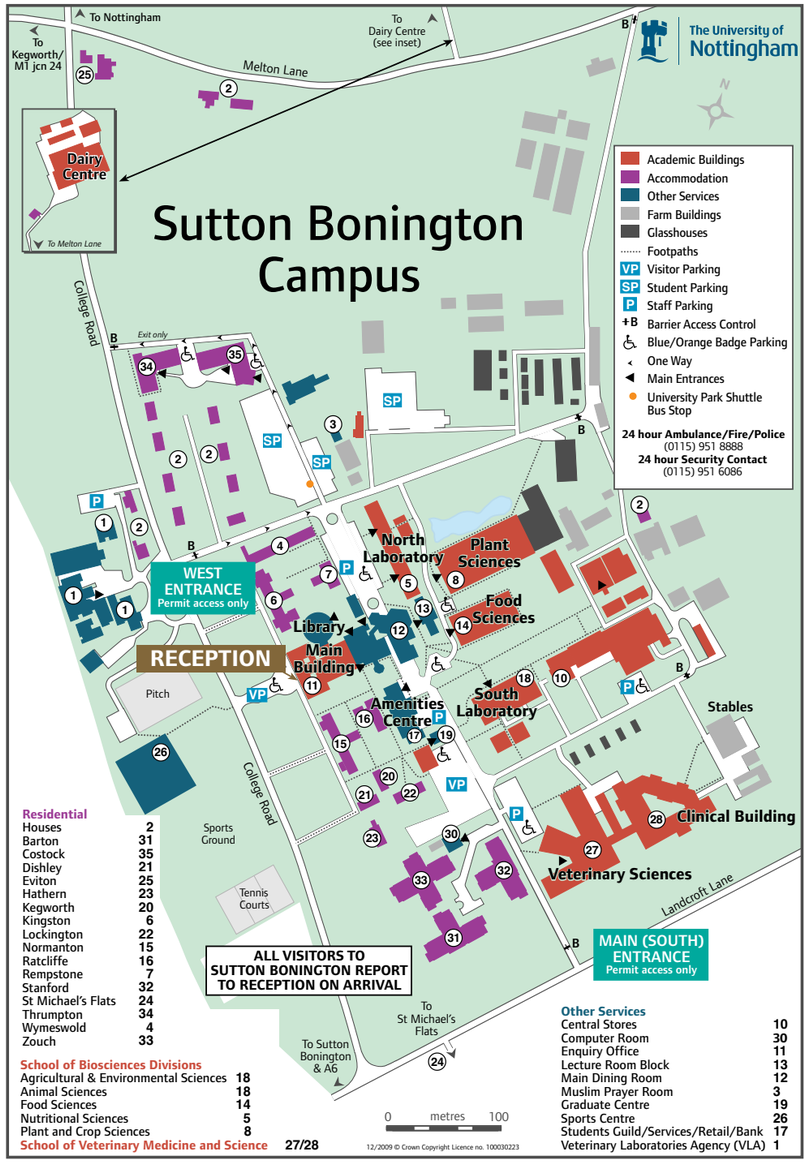
Accidents must be reported to Reception Staff.

Please also take necessary security precautions; lock your door at night, keep valuables secure and out of sight and do not walk alone at night on campus. Do not leave rooms unlocked or property unattended at any time.

Campus Map

Sutton Bonington Campus
University of Nottingham
Sutton Bonington
Leicestershire
LE12 5RD

Telephone: +44 (0)115 951 5151
Website: http://www.nottingham.ac.uk/about/campuses/suttonboningtoncampus.aspx



Conference Information

Registration

Conference attendees are asked to register from 1100 on Sunday 11th July 2010 at the Atrium of the Vet School Building. Here you will also be able to check-in for your accommodation.

On registering, you will receive a name badge with holder and your delegate pack containing the conference programme and abstract book as well as a certificate of attendance.

Opening times:

Sunday 11th July	1100 - 1830
Monday 12th July	0800 - 1800
Tuesday 13th July	0830 - 1600

Name Badges

Please ensure your name badge is worn and visible at all times. This is for security purposes as well as allowing entry to the sessions, poster area and social functions.

Conference Sessions and Catering

The conference sessions will take place in the Vet School Building with parallel sessions taking place in the Lecture Block. Please refer to the full programme for details of exact session/room locations.

Catering and refreshments will be served throughout the conference. Refreshment breaks will be available within the Vet School Building with evening meals being served in the main restaurant.

Poster Sessions

Posters will be on display in the foyer and room A29 of the Vet School Building.

Poster viewing sessions:
Sunday 11th July, 1600 - 1700 (Even poster numbers only)
Monday 12th July, 1600 - 1700 (Odd poster numbers only)

Awards

The Awards Presentation will take place during the Conference Dinner on Monday 12th July. During the dinner, Henry Leese will be presented with his Marshall Medal and all other SRF Award and Prize winners will be recognised.

Please refer to the full programme for details of the award sessions.

Meetings

The Society for Reproduction and Fertility AGM will take place on Monday 12th July from 1300 to 1330 in the Main Auditorium of the Vet School Building.

Social Programme

Sunday 11th July

Drinks Reception
The first night of the conference begins with a drinks reception in the Poster Room from 1830 to 1930.

All delegates and guests are invited to this reception to enjoy a glass of wine together.

SRF Quiz
The "highly competitive but extremely fun" SRF Quiz will follow the drinks reception.

For the evening, a buffet supper will be served from 1930 to 2030 in the main dining room. The bar will be open for delegates requiring drinks after supper.

All delegates and guests are welcome to attend.

Dress code: Casual

Monday 12th July

Pre-dinner Drinks
Pre-dinner drinks will be served in the bar from 1930 for another opportunity to meet socially before the Conference Dinner.

Conference Dinner
The Conference Dinner will be held in the main dining room at 2000.

This formal evening is an excellent occasion to network with colleagues and friends whilst enjoying an evening meal.

During the evening, the SRF Awards Presentation will take place, presenting Professor Henry Leese with the Marshall Medal and recognising all other SRF Prize winners.

Following the meal, guests will be entertained by a Ceilidh band and can dance the night away before enjoying a late drink in the bar. (A cash bar will be available in the main bar.)

All delegates are welcome to attend the **pre-dinner drinks but tickets must be pre-booked and bought to attend the Conference Dinner.**

Dress code: Gentlemen – black tie / lounge suit
Ladies – appropriate evening wear

Programme - Sunday 11th July

TIME	SESSION DETAILS
1200 - 1300	**SRF Council Meeting** (SRF Council Members only)
1400 - 1600	**Symposium I: Molecules of Reproduction** (Main Auditorium A30) **S1** Connections between LH, Amphiregulin Signalling and Ovulation – Malcolm Parker, London, UK **S2** Inhibins and activins revisited – David Robertson, Melbourne, Australia **S3** Interferons and early pregnancy – Tony Flint, Nottingham, UK **S4** IGF peptides and the placenta – Claire Wathes, Hatfield, UK **Chair: Bob Webb**
1600 - 1700	**Poster Session I & Tea/Coffee Break – Even posters only**
1700 - 1830	**Post Doc Scientist Award** (Main Auditorium A30) **O1** Down regulated expression and activity of DNMT1 in early placentae of in vitro produced sheep embryos - A D'Agostino **O2** The Effects of Whole Ovarian Perfusion and Cryopreservation on Endothelial Cell Related Gene Expression in the Ovarian Medulla and Pedicle - VJ Onions **O3** Lipoxins as anti-inflammatory modulators in the human endometrium and first trimester deciduas - LJ Macdonald **O4** The impact of ERβ dependent signalling on vascular function within the human endometrium - E Greaves **Chair: Kurt Sales**
1830 - 1930	**Drinks**
1930 - 2300	**SRF Quiz with Buffet**

Programme - Monday 12th July

TIME	SESSION DETAILS		
0900 - 1100	**Symposium II: Reproduction and development in exotic species** (Main Auditorium A30) **S5** Reproduction and foetal development in elephants – Thomas Hildebrandt, Berlin, Germany **S6** From wild species to domesticant in 30 years: lessons from the reproductive management of farmed red deer – Geoff Asher, New Zealand **S7** In vitro fertilization and embryo transfer in rare felid populations – William Swanson, Cincinnati, USA **S8** Assisted reproductive technologies as applied to canids – Wenche Farstad, Oslo, Norway **Chair: Richard Lea**		
1100 - 1130	**Tea/Coffee Break**		
1130 - 1300	**Oral Session I: Female/male reproduction** (Main Auditorium A30) **O5** Ovine fetal testis development is perturbed by in-utero exposure to a cocktail of chemicals contained in sewage sludge - RG Lea **O6** The role of fibroblast growth factor 2 in regulating angiogenesis during the bovine follicular-luteal transition - M Laird **O7** The role of oxygen availability in ovarian follicle development and function in vitro - JM Connolly **O8** Identifying the role of calcium stores in flagellar motility - JT Morris **O9** Testicular LH-stimulated testosterone production is inhibited by activation of melanocortin 3 receptors (MC3) - K Akindele **O10** A putative role for prokineticins in infection-induced preterm labour - an amnion perspective - TRM Lannagan **Chair: John Parrington & Linsay MacDonald tbc**	**Oral Session II: Ovary/uterus** (Lecture Block A02) **O11** The consequences of germ cell loss on ovarian development in neonatal Dazl knock-out mice - J McNeilly **O12** Toll-like receptor 4 (TLR4) mediates the immune response of epithelial and stromal cells to bacterial lipopolysaccharide in the endometrium associated with pelvic inflammatory disease – IM Sheldon **O13** Exposure to a cocktail of environmental chemicals at critical windows of fetal development: does timing matter to the fetus? - MR Amezaga **O14** Interactions between Prolactin and Estrogenic Hormones in Regulating Uterine Glycogen Metabolism in Mink (Neovison vison) - J Rose **O15** Vectorial secretion of prostaglandins by epithelial cells from the bovine endometrium - SB Price **O16** The effects of a low therapeutic dose of βCG fragment-lytic peptide conjugates on gonadotropin secretion and ovarian function in ewes – E Callery **Chair: Suzannah Williams & Jackie Maybin**	**Oral Session III: Oocyte/embryo** (Lecture Block A03) **O17** Knockdown of p27Kip1 induces the growth of oocytes in mice - M Moniruzzaman **O18** Deletion of the BH3-only gene puma protects oocytes from apoptosis and preserves fertility following anti-cancer therapy-induced DNA damage - JK Findlay **O19** Mechanism of de-novo centriole formation in mouse embryos - K Howe **O20** Epigenetic reprogramming of the porcine germline - SMW Hyldig **O21** Measurement of ATP levels and ATP consumption in the maturing oocyte - CM Dalton **O22** Involvement of selected kinases in cytoplasmic polyadenylation element binding protein dependent translational regulation during maturation of porcine oocytes - P Karabinova **Chair: Henry Leese & Patricia Grasa Molina tbc**
1300 - 1430	**Lunch (SRF AGM (1300 – 1330))**		
1430 - 1600	**SRF Student Prize** (Main Auditorium A30) **O23** Neonatal exposure to aromatisable androgen or estrogen produces a reproductive and metabolic PCOS phenotype in the rat - V Tyndall **O24** STIMulating sperm! STIM-Orai (store-operated) channel subunits are expressed in human sperm and may be a route for rapid Ca2+ influx - K Nash **O25** Transcription factor interactions during lineage segregation in the pig embryo - DA Contreras **O26** Lipoxins are anti-inflammatory modulators in endometrial adenocarcinoma - SP Rajagopal **O27** The expression and regulation of endometrial adrenomedullin (ADM): a novel candidate for post-menstrual repair - JA Maybin **O28** Fetal Programming: Direct and Indirect Fetal Androgenisation Differentially Regulate the Metabolic Phenotype in Adult Sheep - K Hogg **Chair: Mhairi Laird & Leanne Williams**		
1600 - 1700	**Poster Session II and Tea/Coffee – Odd posters only**		
1700 - 1800	**Distinguished Scientist** (Main Auditorium A30) **S9** Polycystic ovary syndrome, reproduction and fertility Professor Steve Franks, London, UK **Chair: Paul Fowler**		
1930	**Pre Dinner Drinks**		
2000	**Conference Dinner**		

Programme - Tuesday 13th July

TIME	SESSION DETAILS
0900 - 1030	**SSR / SRF New Investigator** (Main Auditorium A30) **S10** SSR New Investigator – Wei Yan, Nevada, USA Regulation of Spermatogenesis: from Genetics to Epigenetics **S11** SRF New Investigator – Suzannah Williams, Oxford, UK The regulation of ovarian function and ovulation rate by oocyte-glycoproteins **Chair: DRE Abayasekara**
1030 - 1100	**Tea/Coffee Break**
1100 - 1300	**Symposium III: Animal models: how relevant are they?** (Main Auditorium A30) **S12** Ovarian development in the Zebrafish – C Peng Toronto, Canada **S13** Developmental origins of obesity and hypertension: a role for leptin? – Paul Taylor, London, UK **S14** Animal models of human placentation – Anthony Carter, Odense, Denmark **S15** Prenatally androgenized female Rhesus Monkeys: a possible model for polycystic ovarian disease – Dave Abbott, Madison, USA **Chair: Bob Robinson**
1300 - 1400	**Lunch**
1400 - 1530	**Symposium IV: SRF 60th Anniversary Celebration (Part one)** (Main Auditorium A30) **S16** SRF: an historical perspective – David Whittingham, London, UK **S17** What future for SRF: curiosity or translational driven research in reproduction – David Baird, Edinburgh, UK **S18** A reproductive biologist's view of hormones down the years – Alan McNeilly, Edinburgh, UK **Chair: Paul Fowler**
1530 - 1600	**Tea/Coffee Break**
1600 - 1730	**Symposium IV: SRF 60th Anniversary Celebration (Part two)** (Main Auditorium A30) **S19** The Journey of ART until now – Peter Brinsden, Cambridge, UK **S20** Transgenics: invaluable to reproductive biology – Harry Charlton Oxford, UK **S21** Sperm biology in the 21st century – Harry Moore, Sheffield, UK **Chair: Tony Flint**
1730 - 1745	**Closing Ceremony** (Main Auditorium A30) DRE Abayasekara

Biographies

Marshall Medallist – Henry Leese

Henry Leese graduated in Physiological Chemistry from Reading University. His PhD (Imperial College London) was on the effect of diabetes on glucose transport and metabolism by small intestine and he continued to work on nutrient absorption and metabolism as a post doc at York University. This was followed by a year at the Swiss Federal Institute of Technology in Zurich studying the enzyme lactase. He then returned to York and changed research direction to examine secretion by the female reproductive tract, and following a sabbatical in John Biggers' lab (Harvard USA) in 1980, began work on metabolism in early mammalian embryos. He was given a Personal Chair at York in 1997. He has been awarded Honorary Fellowships by the Association of Clinical Embryologists (ACE: 2002) and British Fertility Society (BFS: 2010) and was made a Fellow of the Royal College of Obstetricians and Gynaecologists (FRCOG *ad eundem*) in 2003. From 1998-2002 he was a member of the Human Fertilisation and Embryology Authority. He is co-founder and Editor-in-Chief of *Human Fertility*, produced on behalf of the BFS, the ACE, the British Andrology Society, the British Infertility Counselling Association and the Royal College of Nurses Fertility Nursing Group reflecting his belief that issues in reproductive biology and medicine benefit from a multidisciplinary approach. To this end, he organised the first joint meeting of UK Fertility Societies, including the, then, SSF in York in 1997. He has supervised 25 PhD students and teaches reproductive biology, human nutrition and metabolism to undergraduates and postgraduates in biology and medicine.

Distinguished Scientist – Steve Franks

Stephen Franks trained in Internal Medicine and Endocrinology. He is Professor of Reproductive Endocrinology at Imperial College Faculty of Medicine (University of London) and Consultant Endocrinologist at St Mary's and Hammersmith Hospitals, London. His initial research was in disorders of human prolactin secretion under the supervision of Howard Jacobs and John Nabarro at the Middlesex Hospital, London. He then spent 2 years as a postdoctoral research fellow in reproductive endocrinology in the lab of Dr Fred Naftolin at McGill University, Montreal (funded by an MRC Travelling Fellowship) before returning to resume his training in endocrinology with Bill Hoffenberg in Birmingham. He is a former Chairman of the Society for Endocrinology (UK), currently sits on their Clinical Committee and is a long-term member of the Society for Reproduction and Fertility. He is a Fellow of the Academy of Medical Sciences and holds an honorary doctorate from the University of Uppsala, Sweden. He is a former editor of Clinical Endocrinology. He has both clinic and laboratory based programmes of research in the field of normal and disordered function of the hypothalamic-pituitary-ovarian axis. He has a major interest in polycystic ovary syndrome, which is not only the commonest cause of anovulatory infertility but is also a major risk factor for development of type 2 diabetes. His research includes investigation of disorders of follicle development, the mechanism(s) of anovulation, and of the characteristic metabolic abnormalities; it focuses particularly on the interaction between genetic and environmental factors in the aetiology of the syndrome.

Biographies

SSR New Investigator – Wei Yan

Dr Wei Yan obtained his MD from China Medical University (in 1990) and his PhD from University of Turku, Finland (in 2000). After post-doc training at Baylor College of Medicine, Houston, TX, Dr Yan started his own lab in the University of Nevada School of Medicine in 2004. Dr Yan is currently an Associate Professor of Physiology, Molecular and Cellular Biology. He has published >60 peer-reviewed research articles, many of which appeared in prestigious journals, including *Nature Genetics, PNAS, Nucleic Acids Research, RNA, Molecular and Cellular Biology, Developmental Biology,* etc. Dr Yan received the 2009 Nevada High Education System Regents' Rising Researcher Award and the 2009 SSR Young Investigator Award. He has served as an ad-hoc reviewer for >30 biomedical journals and in two NIH study sections. Dr Yan is currently funded by two major NIH grants. Dr Yan's research is focused upon spermiogenesis-specific genes and testicular small non-coding RNAs. He believes that the malfunction of late spermiogenesis-specific genes is involved in many forms of sperm defects in idiopathic male infertility patients. Since these late spermiogenesis-specific genes and/or gene products are unique to male haploid germ cells, they may serve as good male non-hormonal contraceptive targets. His lab was also one of a few, which first performed extensive cloning and expression profiling analyses on testicular small non-coding RNAs. Functional characterization of X-linked miRNAs and testis-specific miRNAs are underway, which will elucidate the roles of testicular small RNAs in spermatogenesis.

SRF New Investigator – Suzannah Williams

Suzannah Williams obtained her Honours degree in Zoology from the University of Aberdeen (1995). As an undergraduate she was inspired to follow an academic career in reproductive biology by Dr Morley Hutchinson and Prof Paul Fowler. Dr Williams obtained her PhD investigating nutritional regulation of ovulation rate in sheep from the Royal Veterinary College, London supervised by Prof Rex Scaramuzzi. During her PhD, she worked with Dr Diarmuid O'Callaghan at University College Dublin and Prof Graeme Martin at the University of Western Australia. As a result, after her PhD, Dr Williams worked with Prof Martin funded by a Wain Postdoctoral Fellowship (1999) and a Lalor Foundation Fellowship (2000). In 2002, she joined Dr Pamela Stanley's laboratory at the Albert Einstein College of Medicine, New York. In Dr Stanley's laboratory, Dr Williams investigated the role of the oocyte on fertility using mice with oocyte-specific deletion of glycosyltransferases. Studies using these mice generating oocytes lacking specific glycans were used to refute long standing sperm-zona binding hypotheses and resulted in an invitation to speak at the 2007 Gordon Research Conference "Fertilization and the Activation of Development". Further investigation has revealed novel roles for the oocyte in the regulation of follicle function, ovulation rate and ovarian failure. Dr Williams joined the University of Oxford as a Lecturer in 2006 and has since established her own research programme with the central aim of investigating the mechanisms involved in the regulation of fertility by the oocyte. Dr Williams is funded by the MRC.

Biographies

Symposium I: Molecules of reproduction

Malcolm Parker

Malcolm Parker is a Molecular Biologist with research interests in nuclear receptor signalling in endocrine related cancers, metabolism and reproduction. He worked for many years at Imperial Cancer Research Fund where his laboratory investigated androgen and oestrogen action in prostate and breast cancer, respectively. During this time his group discovered a number of transcription cofactors for nuclear receptors that they called RIP140 and RIP160. In 2001 Malcolm Parker moved to Imperial College where he was appointed Head of the Institute of Reproductive and Developmental Biology. He has continued to focus on the function of RIP140 in female fertility and in energy homeostasis.

David Robertson

David Robertson received his Doctorate of Philosophy in 1978 at Stockholm University, (Supervisor Egon Diczfalusy) returning to Australia to undertake postdoctoral work with David de Kretser, Monash University, Australia. In 1989, he joined Prince Henry's Institute of Medical Research, Clayton, Victoria.

He currently holds positions of Senior Scientist, Prince Henry's Institute of Medical Research, Career Scientist under the NHMRC Research Fellowship Scheme of Australia and Adjunct Associate Professor, Dept Obstetrics and Gynaecology, Monash University.

His research interests have centred on the isolation, characterisation and measurement of reproductive hormones and factors including inhibin, follistatin, gonadotrophins and the elucidation of their roles in reproductive endocrinology of both the male and female. His current interests include studies on the role of inhibins in the hormonal regulation of FSH (including the menopause transition) and the use of proteomics to identify tissue and plasma biomarkers in infertility and cancer. He has close ties with industry in the area of inhibin and related molecules with the development of specific assays for the diagnosis of ovarian cancer. He is an inventor on 4 patents in this area.

Biographies

Tony Flint

Tony Flint enrolled at the University of St Andrew's (Queen's College Dundee) in 1962 to read Physiology, but after just 6 lectures transferred to Biochemistry. His undergraduate project, on ascorbic acid uptake by luteal tissue, stimulated an interest in reproductive physiology, and after graduating in 1966 he went to Bristol to do a PhD with Philip Randle and Dick Denton, on glucose metabolism in the ovary. In 1969 he went to London, Ontario for a postdoc, jointly in the departments of Physiology and Obstetrics and Gynecology, with David Armstrong. He returned to the UK in 1972 to Cardiff to work with Alec Turnbull and later moved with him to Oxford, on an MRC programme grant studying preterm labour. While there Tony discovered how fetal cortisol controls the onset of labour in sheep, and showed that this mechanism did not apply in women. In 1977 he moved to the Institute of Animal Physiology at Babraham, and in 1987 became Director of the Institute of Zoology at the Zoological Society of London. During this period he showed how corpus luteum secretion of oxytocin and blastocyst interferons are involved in the maternal recognition of pregnancy in ruminants.

Despite giving up physiology at an early stage, Tony was appointed to the Chair of Animal Physiology at Nottingham in 1993, from which he retired in 2009. He is currently Editor-in-Chief of Reproduction, Fertility and Development, and remains as an Emeritus Professor at Nottingham.

Claire Wathes

Claire Wathes graduated with a 1st class BSc in Biological Sciences from the University of Birmingham in 1974 then studied for a PhD under Eric Lamming at the University of Nottingham. Here she developed the use of the milk progesterone test to study fertility in dairy cows. Following 2 years experience as a postdoctoral scientist at the Babraham Institute, she moved to the Department of Anatomy at the University of Bristol from 1979-1990 first as a lecturer, then as a Reader, being awarded a DSc in 1990. She then returned to the Babraham Institute as a project leader for 4 years before taking up her present post as Professor of Veterinary Reproduction at the Royal Veterinary College, London. She was awarded the Research Medal of the Royal Agricultural Society of England in 2006.

Her main research focus is on farm animal reproduction. She has a longstanding interest in the causes of infertility in dairy cows and has also worked extensively on various aspects of ovarian function, early pregnancy and placental development in cattle and sheep. The current main emphasis of her studies is in understanding the metabolic signals which influence fertility.

She has served on a number of BBSRC committees and was Chair of the steering committee for ARK-Genomics from its establishment in 2000 until 2007. She has also maintained an active participation on a wide variety of grants and science strategy review committees and journal editorial boards.

Biographies

Symposium II: Reproduction and development in exotic species

Thomas Hildebrandt

Employment: March 1997 – present: Head of Department Reproduction Management, Institute for Zoo and Wildlife Research (IZW), Berlin
January 1995 – February 1997: Head, Research Group Ultrasound, IZW
February 1992 – 1994: Scientific researcher, IZW
1985 – 1986: Pathology assistant, veterinary pathology of the faculty of veterinary medicine, Humboldt-University Berlin
1984 – 1985: Farm work, dairy cattle farm
1982 - 1984: Army

Scientific Awards: Best Research Presentation Established Investigators, Int. Symp. Canine and Feline Reproduction, Vienna, July 2008
Appointment to Veterinary fellow of the Zoological Society of London, 2004
Appointment to Fellow of the Zoological Society of San Diego, 2001
Appointment to Research Associate of Smithsonian Institution, National Zoological Park, 2000
Honourable Award Ultrasound in Elephants at National Zoological Park in the Photo Contest in category “Science” for the 150 anniversary Smithsonian Institution (23.05.1996)
Short - Term Visitor Award at the Smithsonian Institution's National Zoological Park (Between July 1 and September 30, 1995)
National Scholarship for best study results (1988-1990)
5 Poster Awards

Geoff Asher

BSc (1977) and MSc(Hons) (1979) in Zoology and Botany, Victoria University, Wellington. PhD (1986) in Animal Sciences, Lincoln University, Canterbury.
1980-1993: Scientist at the Ruakura Animal Research Station (Research Division, Ministry of Agriculture and Fisheries), Hamilton, NZ.
1993-present: Senior Scientist and Programme Leader at the Invermay Agricultural Centre (AgResearch Ltd), Mosgiel, NZ.
Refereed publications: 94 - Other publications: 180
Research: Reproductive physiology and productivity of farmed deer species; including red deer, fallow deer and Wapiti.

I have had a 30-year involvement with the NZ deer farming industry, principally investigating reproductive productivity. I am presently the programme leader of ‘The Venison Supply Systems Programme” administered through DEEResearch Ltd, which is a joint venture partnership between Deer Industry New Zealand (DINZ) and AgResearch, which invests $NZ2 million per annum into venison productivity research. Current personal research projects include determinants of female puberty and high-country (sub-alpine) deer systems.

Biographies

William Swanson

Dr William Swanson is the Director of Animal Research at the Center for Conservation and Research of Endangered Wildlife (CREW), based at the Cincinnati Zoo and Botanical Garden. His educational background includes a BS in Zoology from the University of Texas, a DVM from Texas A&M University and a PhD in Animal Science from Louisiana State University. Following graduate school, Dr Swanson completed a two-year post-doctoral fellowship at the Smithsonian Institution's National Zoological Park in Washington DC and was employed for three years as a gamete biologist at the Smithsonian’s Conservation and Research Center. In 1997, he assumed his current position at the Cincinnati Zoo and Botanical Garden.

Over the past 20 years, his research efforts have focused primarily on investigating the reproductive biology of domestic cats and endangered wild cats to improve their captive management and conservation. His studies, conducted in the United States and in 18 foreign countries, have involved research with 30 wild felid species and 24 domestic cat models of human hereditary disease. Dr Swanson also serves as Co-Chair of the Felid Taxon Advisory Group for the Association of Zoos and Aquariums (AZA), helping to oversee population management programs for the 18 wild cat species maintained in 220 AZA-accredited North American zoos and aquariums.

Wenche Farstad

Graduated in 1979 from the Norwegian School of Veterinary Science, Oslo, DVM (cand.med.vet.). She obtained a PhD (dr. scient.), thesis on canine reproduction 1984, and a free level doctorate degree (dr.med.vet.), thesis on fox reproduction in 1993. She became associate professor of reproduction in 1985, and full professor from 1995 at the Norwegian School of Veterinary Science. She was instrumental in the foundation of the European College of Animal Reproduction and the European Veterinary Society for Small Animal Reproduction and served as Board member of both for several years. She is now a lifetime honorary member of EVSSAR and founding Diplomate of ECAR. She also serves at the advisory Board of the Institute for Breeding Rare and Endangered African Mammals (IBREAM). Her clinical and research interest are animal reproduction and biotechnology and she has published 145 scientific and tutorial articles within animal reproduction and been invited speaker to numerous international congresses particularly relating to her main interest in canine reproduction. Privately, she has bred English setters and English springer spaniels under the prefix Streamside for 20 years. She is a field trial judge and is member of several national and international canine societies such as The Kennel Club (UK).

Biographies

Symposium III: Animal models: how relevant are they?

Chun Peng

Dr Chun Peng is a professor of Biology at York University. She obtained her PhD from University of Alberta in 1993. Following postdoctoral training at the Department of Obstetrics and Gynecology, University of British Columbia, she joined the Department of Biology at York University in 1995. She was promoted to Associate Professor in 2000 and Full Professor in 2007.

Dr Peng's group is interested in the function, regulation and signaling mechanism of the transforming growth factor-beta (TGF-beta) superfamily in the ovary and placenta. Major research programs in her lab include: 1) regulation of zebrafish follicle development and oocyte maturation by activin, TGF-beta and BMP-15; 2) Role of Nodal and microRNAs in placental development and pregnancy complications; and 3) Integrative functions of Nodal, ALK7, cyclin G2 and microRNAs in ovarian tumorigenesis.

Dr Peng research is supported by Natural Science and Engineering Research Council (NSERC) and Canadian Institute of Health Research (CIHR). She has received many awards during the course of her career, including a Women's faculty award from NSERC, a Premier's research excellent award, and a mid-career award from CIHR and Ontario Women's Health council.

Paul Taylor

Dr Paul Taylor is a cardiovascular physiologist, with an interest in the early life origins of cardiovascular and metabolic disease. In 1994 he obtained a PhD in Physiology from United Medical & Dental Schools, London University investigating the mechanisms underlying the vascular complications of diabetes. This was followed by postdoctoral training in the Vascular Biology Unit, Boston University USA, and Department Clinical Pharmacology, St George's Hospital Medical School, London. A further post-doctoral position within the Maternal & Fetal Research Unit at King's College London combined interests in the role of nutrition in cardiovascular health and disease, with the emerging field of fetal programming. In 2005, Dr Taylor was appointed Lecturer in Developmental Programming at Kings College London within the Division of Reproduction & Endocrinology and Senior Lecturer in 2008.

Dr Taylor's research interests include the 'developmental programming' effects of maternal nutrition and the hormonal environment in obese and diabetic pregnancy on the offspring's future cardiovascular and metabolic development. The goal of the Developmental Programming Research Group is "To understand the consequences of maternal obesity in pregnancy on the future health of the child". Specifically, the group aim to investigate the physiological processes and the cellular and molecular mechanisms whereby a baby's exposure to an aberrant hormonal environment in pregnancy and lactation gives rise to increased risk of obesity and cardiovascular disorders in later life. Recent publications in the journal Hypertension provide evidence for the sympathetic origins of hypertension secondary to maternal obesity.

Biographies

Anthony Carter

Anthony M Carter is Reader in Physiology at the University of Southern Denmark. He was educated at Latymer Upper School and Magdalene College Cambridge and did his postgraduate work at the Department of Obstetrics and Gynaecology of the University of Lund, Sweden. He moved to Denmark in 1976. He has served as Dean of Medicine at the University of Southern Denmark and has been a Visiting Professor at the Universities of Toronto and Western Ontario. He is Adjunct Curator of the Harland W Mossman Collection at the University of Wisconsin Zoological Museum. Anthony Carter's research interests include uteroplacental circulation, placental gas exchange, foetal endocrinology and the control of foetal growth. In recent years he has focused on comparative studies of placentation and theories of placental evolution. Anthony Carter is Editor of Trophoblast Research, which appears as a supplement to Placenta.

Dave Abbott

David Abbott received his PhD in Zoology from the University of Edinburgh in 1979 before taking up a postdoc in the Endocrinology-Reproductive Physiology Training Program at the University of Wisconsin, Madison (79-81). This was followed by a postdoc in the Dept. of Anatomy at the University of Cambridge (81-83), prior to appointment as Head of the Behavioral Physiology Unit at the Institute of Zoology, Royal Zoological Society of London (84-90). He moved to his current position, Professor in the Dept. of Obstetrics and Gynecology and Wisconsin National Primate Research Center, University of Wisconsin, Madison in 1991. His initial focus on behavioural, neuroendocrine and sensory mechanisms of social dominance regulation of ovulatory function in female mammals, particularly marmoset monkeys and African naked mole-rats, has progressed into more translational studies with an emphasis on developmental programming of polycystic ovary syndrome (PCOS). Together with clinical and basic science collaborators, the Abbott lab has led the exploration of fetal androgen excess as a developmental origin of PCOS. Studies in a nonhuman primate model demonstrated how discrete fetal androgen exposure permanently alters female reproductive and metabolic physiology to resemble much of the pathophysiology found in women with PCOS. The current focus involves determining epigenomic and metabolomic mechanisms of developmental programming of PCOS, with an emphasis on metabolic and pancreatic pathophysiology.

Biographies

Symposium IV: SRF 60th anniversary celebration

David Whittingham (Photo and biography unavailable)

David Baird

David Baird(DSc,MD, FRSE FmedSci CBE)) was educated at the Universities of Aberdeen, Cambridge and Edinburgh before training in endocrinology, obstetrics and gynaecology in Edinburgh and London .From 1977 to 1985 he held the Chair of Obstetrics and Gynaecology at the University of Edinburgh until being appointed MRC Clinical Research Professor of Reproductive Endocrinology.

His research interests include both basic and applied in the field of reproductive medicine from infertility to contraception. Amongst his research achievements have been the identification of PGF2α as the luteolytic hormone in sheep and many other species; the role of prostaglandins in menstruation and its disorders; the development of antigestogens (RU486) as medical abortifacients and contraceptives; a unique method of preserving reproductive function in women undergoing chemo/radiotherapy for cancer by cryopreservation and autotransplantation of the ovary. His current research involves investigating the mechanism by which mutations in the BMPR1B receptor ("fecundity gene") results in multiple ovulations in sheep.

He has received a number of Prizes and prestigious awards from professional societies including Clinical Endocrinology Medal and Dale Medal from the Society of Endocrinology, Lecture and Marshall Medal from the Society for Study of Fertility, Honorary Member of ESHRE, Eardley Holland Medal and Fletcher Shaw Lecture and Medal, Royal College of Obstetricians and Gynaecologists, Life Time Achievement Award Reproductive Ruminant Society. He is Past Chairman of SSF and Honorary Past President of the British Fertility Society.

Alan McNeilly

Alan is a graduate of Sutton Bonington and gained a PhD from Reading and a DSc from Edinburgh universities. He became fascinated by reproductive biology, particularly the endocrine control of female reproduction during his undergraduate years at SB, and transferred top researching clinical reproductive problems during his time as a postdoc at Barts hospital before a year's sabbatical in Winnipeg working on prolactin biology. He achieved a lifetime ambition of working with Roger Short and David Baird in the MRC reproductive Sciences Unit in Edinburgh in 1976 where he has remained ever since. His research has encompassed a number of ventures into the regulation of ovarian function from how the pituitary manages to produce FSH and LH in completely different manner from the same cell type, through the regulation of reproduction by alterations in GnRH pulse frequency modulation, particularly in breastfeeding women, to the local regulation of follicle development by activins, inhibin and FSH. Latterly the discovery of the essential role of DAZL in maintaining oocyte competence, without which ovarian tumors form, and confirming GDF9 as being an essential component for follicle development in sheep has fuelled further recent work into the regulation of ovulation rate in sheep and how alterations in the microvasculature may influence this selection of dominant follicles with viable oocytes. Of particular importance has been the many collaborations with excellent scientists and friends around the world in pursuing his research aims, from development of assays through to new animal models and application to human reproductive medicine.

CONFERENCE **2010**

Society for Reproduction and Fertility

Biographies

Peter Brinsden

Peter Brinsden was born in Peking, China in 1940. After attending schools in Canada, China, Hong Kong and England, he entered King's College, University of London in 1959, followed by St George's Hospital, London, from where he qualified MB BS in 1966. After serving as a Medical Officer in the Royal Navy between 1966 and 1982, he left the Navy in the rank of Surgeon Commander.

Peter Brinsden became a Consultant Gynaecologist in 1978, with his main interest in infertility. He served at Bourn Hall Clinic with Mr Steptoe and Professor Edwards in 1985 and then joined Professor Ian Craft as Deputy Medical Director of the Wellington Hospital IVF Unit, at that time the largest IVF Unit in the world. He was appointed Medical Director of Bourn Hall Clinic, Cambridge, in December 1988, following the death of Mr Steptoe. He is now Consultant Medical Director. He travels widely for teaching and consultancy work, and holds four Honorary or Visiting Professorships in PR China. In April 2009 he was elected President of the British Fertility Society.

He is the author or co-author of more than 100 publications on Infertility and Assisted Conception, the author of 46 book chapters, co-editor of "The Infertility Manual" and "A Handbook of Intrauterine Insemination", and is editor of three editions of "A Textbook of In Vitro Fertilisation and Assisted Reproduction".

His personal interests include sailing, scuba diving, photography and his grandchildren.

Harry Charlton (Photo and biography unavailable)

Harry Moore

Harry Moore holds the Chair of Reproductive Biology in the Department of Biomedical Science at the University of Sheffield where he co-directs the Centre for Stem Cell Biology. Harry was an undergraduate at Reading University where his tutor was Geoff Waites, one of the early members of the SRF and who steered him into a lifelong interest in sperm function. His Ph.D. was on cryopreservation of boar sperm at the Institute of Animal Health, Compton. Having decided not to become a pig farmer, he took up a Ford Foundation postdoctoral fellowship at Cornell Medical College investigating sperm maturation and fertilisation events with his mentor Michael Bedford who ideas on the co-evolution of gametes have greatly influenced him ever since.

On returning to the UK, he was Zuckerman Research Fellow at the Zoological Society of London continuing research on gamete development and artificial breeding for endangered mammals such as the Puma and Giant Panda. In 1992, he was appointed to his current position and since 2001 established the Centre for Stem Cell Biology working on the generation and application of human embryonic stem cells. His laboratory discovered the phenomenon of sperm cooperation as 'sperm trains' in the wood mouse, and has derived human trophoblast stem cells and post-meiotic germ cells from embryonic stem cells. With his colleague Tim Birkhead, he organises the Biology of Spermatozoa meeting every two years which discusses the evolution of spermatozoa in all its forms.

Sunday 11th July 2010

Symposium I: Molecules of reproduction
Main Auditorium
1400 – 1600

Speaker abstracts

Notes

S1 Connections between LH, Amphiregulin Signalling and Ovulation

J Nautiyal[1], J Steel[1], J Richardson[2], MM Rosell[2], E Nikolopoulou[1], R White[1] & **M Parker**[1]
[1]Institute of Reproductive and Developmental Biology, Faculty of Medicine, Imperial College London, London, UK; [2]Department of Molecular and Cellular Biology, Baylor College of Medicine, Houston, Texas, USA

Introduction: Nuclear receptors regulate reproductive function by activating or repressing transcription of gene networks in target cells in response to fluctuating hormones and growth factors. We have identified transcriptional cofactors that are recruited to nuclear receptors, remodel chromatin and thereby regulate gene transcription. One cofactor we identified called RIP140 is essential for ovulation.
Methods: The role of RIP140 in ovulation was characterised in mice and its function determined in cumulus oocyte complexes (COCs) and granulose cells. Target genes were identified by expression profiling and its molecular mechanism of action elucidated using transfected reporter assays and chromatin immunoprecipitation (ChIP).
Results and discussion: Cumulus expansion and follicular rupture in response to the LH surge does not occur in RIP140 null mice. Gene expression profiling indicates that amphiregulins and a number of matrix protein and proteases responsible for cumulus expansion are not expressed in the absence of RIP140. The ability of COCs from RIP140 null mice to undergo expansion
In vitro can be restored by amphiregulin treatment suggesting that it is a direct target for RIP140. Reporter assays and ChIP experiments indicate that RIP140 promotes transcription from the amphiregulin promoter by stimulating CREB and c-Jun family members.
Thus we conclude that RIP140 is functioning as a transcription coactivator for CREB in the ovary. Paradoxically while RIP140 functions as a coactivator for certain transcription factors it functions as a corepressor in metabolic tissues.

S2 Inhibins and Activins Revisited

DM Robertson
Prince Henrys Institute of Medical Research, Clayton, Victoria, Australia

Inhibins and activins are members of the TGFβ superfamily which play critical roles in the regulation of fertility. Inhibins A and B are produced by the gonads and consist of a common α subunit and either βA or βB subunits. Activins are dimers of the β subunits which regulate diverse functions by binding to two types of serine:threonine kinase receptors (activin Type I and II receptors). Inhibin's action is inhibitory based on its association with an ancillary binding protein, betaglycan which together with the Type II receptor inhibits the formation of a signalling complex with the Type I receptor. Inhibin's antagonism is based on its ability to reduce the levels of either betaglycan or the Type II receptor binding sites and thus limit the signalling response. Recent studies have suggested that inhibin antagonises the actions of activin and other TGFβ ligands including TGFβ2, BMP2,4,7 and GDF5. Thus the potential roles of inhibin are not constrained to the reproductive system but relate to the tissue specific expression of ligand, betaglycan and Type II receptor. In the pituitary it is believed that the primary inhibitory role of inhibin is to antagonise activin-stimulated FSH synthesis and secretion. Alternatively, its action in bone may be to inhibit BMP function.
Our recent studies show that there are additional levels of inhibin action in these systems. Results from our laboratory indicate that there are differences in biological activities between inhibin A and B providing another level of regulation of FSH and thus fertility. This presentation will present an overview of our current understanding of inhibin action with a particular emphasis on the regulation of FSH.

Notes

S3 Interferons and early pregnancy

A Flint
School of Biosciences, University of Nottingham, Sutton Bonington Campus, Loughborough UK

The maternal recognition of pregnancy represents an important stage in gestation. Luteal regression is blocked, the corpus luteum of the cycle is maintained, and maternal progesterone secretion continues in order to stimulate endometrial gland secretion of the nutrients necessary for growth of the conceptus. In ruminants, in the absence of conception, luteal regression occurs as a result of the episodic secretion by the endometrium of prostaglandin F2α. Each episode of prostaglandin F2α is driven by an episode of oxytocin secreted by the corpus luteum. Oxytocin secretion by the corpus luteum is stimulated by prostaglandin F2α, and prostaglandin F2α secretion by the endometrium is stimulated by oxytocin, so there is a positive feedback relationship between them. The response of the endometrium to oxytocin depends on availability of the oxytocin receptor, which is expressed for a short period at the end of each non-pregnant cycle. In pregnancy the endometrial expression of the oxytocin receptor is blocked, so there are no episodes of prostaglandin F2α secretion, and no luteal regression. The compound responsible for blocking expression of the oxytocin receptor is an interferon-tau, which is secreted by the conceptus. This family of trophoblast-specific interferons are expressed at the time of embryo elongation, before attachment and implantation of the conceptus, when they represent the most abundant protein expressed. After about a week during which the conceptus secretes interferon-tau, luteal oxytocin secretion ceases, episodes of prostaglandin F2α secretion no longer occur, and interferon-tau expression stops. Ruminants lacking luteal regression, such as the roe deer (Capreolus capreolus) do not require a maternal recognition of pregnancy signal, and do not produce interferon-tau.

S4 IGF peptides and the placenta

C Wathes
Royal Veterinary College, Reproduction and Development Group, Hatfield, UK

The size and efficiency of the placenta and the metabolic status of the dam all influence nutrient transfer to the fetus and hence play a key role in ensuring a successful pregnancy outcome. Following an initial growth phase, there is continual remodelling of the placenta and its associated vascular supply during pregnancy. Both stages are regulated by a complex signalling dialogue between maternal and fetal tissues. IGF2 is the main peptide synthesised in the placenta itself, but IGF1 produced either locally or obtained via the systemic circulation is also important. The key role of IGF2 in placental development was shown by deletion of placental igf2 in mice, which results in severe growth restriction. In vitro studies demonstrate clearly that IGF1 and IGF2 can induce trophoblast proliferation and influence trophoblast migration, placental angiogenesis and nutrient transfer. This may be achieved through interaction with numerous other growth factors (eg leptin, TGF-β, VEGF, TNFα, insulin) to alter placental phenotype via modulation of transcription factor activity. IGF receptors are detectable in the embryo from the 2 cell stage onwards, suggesting that IGFs secreted by the oviduct and endometrium influence early development even before attachment occurs. The presence of IGF1R on endometrial glands also supports a role for maternal IGFs in the control of histotrophic secretion. However the identity of the main IGF receptor mediating IGF2 activity in the mature placental remains controversial. Although IGF2 can signal through both the IGF1R and the INSR, there is strong evidence that another receptor type is more important in the placenta. This might be a hybrid IGF1R/INSR and/or post-membrane signalling through the IGF2R. Locally produced IGF binding proteins also play an important role by regulating IGF bioactivity within placental tissues. In particular IGFBP production by the maternal endometrium probably controls the degree of trophoblast invasion.

Sunday 11th July 2010

Post Doc Scientist Award
Main Auditorium
1700 - 1830

Presenter abstracts

Notes

O1 Down regulated expression and activity of DNMT1 in early placentae of in vitro produced sheep embryos

A D'Agostino[1], P Toschi[1], A Fidanza[1], F Zacchini[1], F Monaco[2], P Loi[1] & G Ptak[1]
[1]Comparative Biomedical Science, University of Teramo, Teramo, Italy; [2]Istituto Zooprofilattico Sperimentale dell'Abruzzo e del Molise, Teramo, Italy

Assisted reproduction technologies (ART) are associated with an increased risk of epigenetic defects causing aberrant placental and embryonic development. DNMT1, DNA methyltransferase most abundant and widely expressed in mammals, is essential for the maintenance of the methylation pattern of imprinted and non-imprinted genes in the post-implantation period. In this work we test the hypothesis that DNMT1 is deregulated in placentae obtained by ART and this deregulation can cause altered expression of placental genes i.e. aberrant placental development. To do this, we quantify (1) the expression of DNMT1 at protein and mRNA level, (2) enzymatic activity of DNMT1 as well as (3) the expression of 6 angiogenetic factors and 5 imprinted genes in early chorioallantoic tissues (CA) obtained from sheep embryos obtained by ART. ART embryos were produced entirely in vitro by oocyte maturation and fertilization and 6 days of embryo culture followed by transfer to synchronized recipient sheep. Control CA were obtained by natural mating. CA were recovered from recipient sheep at days 20, 22, 24, 26 and 28 of pregnancy. Semi-quantitative (Western blotting) and quantitative (ELISA, RT-PCR) analysis confirmed that DNMT1 (protein and mRNA) expression is constantly down-regulated in CA obtained from ART embryos from day 20 till 28 of gestation. The enzymatic activity of DNMT1 in this period was also constantly lower ($P<0.005$) in CA obtained from ART embryos. Further analysis revealed generally lower ($P<0.05$) level of mRNA expression of angiogenetic factor genes (3 of 6) and imprinted genes (3 of 5) involved in early placental development. These results show down regulated expression and low enzymatic activity of DNMT1 in sheep CA during the establishment of pregnancy. DNMT1 insufficiency could be likely responsible for defects in placental development of ART embryos. Such placental defects could be the primary cause of early pregnancy loss.

O2 The effects of whole ovarian perfusion and cryopreservation on endothelial cell related gene expression in the ovarian medulla and pedicle

VJ Onions[1], R Webb[2], HM Picton[3] & BK Campbell[1]
[1]School of Clinical Sciences, University of Nottingham, Nottingham, UK; [2]School of Biosciences, University of Nottingham, Loughborough, UK; [3]Leeds Institute of Genetics, Health and Therapeutics, University of Leeds, Leeds, UK

Introduction: Fertility preservation by whole ovarian cryopreservation requires successful cryopreservation of both the ovary and its vascular supply. Our previous work has indicated that both perfusion and cryopreservation of the ovary and vascular pedicle can lead to a loss of subsequent ovarian function. This study determined whether this loss was caused by effects on the vascular supply and used targeted arrays to assess the acute effects of blood perfusion, alone or in combination with cryopreservation, on endothelial cell related gene expression within the ovarian medulla and pedicle.
Methods: The ovarian arteries of ovine reproductive tracts were cannulated and the ovary and pedicle dissected. Left ovaries (n=4) were perfused with heparinised blood and flushed with Ringers. Right ovaries (n=4) were cryopreserved using a standard slow-freezing protocol and following thawing were perfused with heparinised blood and flushed with Ringers. Samples of medulla and pedicle from each treatment were snap frozen. cDNA was synthesised and a customised real-time PCR array used to assess the expression of 32 genes. *eNOS* and *ET-1* expression was also analysed using real-time PCR. Data were compared with non-perfused controls (n=4).
Results and Discussion: In the medulla, perfusion alone resulted in downregulation in expression of the pro-apoptotic gene *CASP6* and *THBS1*; associated with angiogenesis and platelet function. Following cryopreservation, *eNOS*, *ET-1*, *EDNRA* and *Bcl-2* expression was significantly ($P<0.05$) downregulated; representing vascular tone and apoptotic pathways. In the pedicle, neither perfusion nor cryopreservation significantly affected expression of the genes studied. In conclusion, this study has identified a number of endothelial cell related genes expressed in the medulla which are acutely affected by both cryopreservation and perfusion, thus supporting the hypothesis that both these interventions have deleterious effects on endothelial cell function. These results will facilitate the development of more focussed approaches to overcome these deleterious effects.

(Supported by MRC funding).

Notes

O3 Lipoxins as anti-inflammatory modulators in the human endometrium and first trimester decidua

LJ Macdonald[1], SP Rajagopal[1], SC Boddy[1], HOD Critchley[2], KJ Sales[1] & HN Jabbour[1]
[1]Human Reproductive Sciences Unit, Medical Research Council, Edinburgh, UK; [2]Reproductive and Developmental Sciences, University of Edinburgh, Edinburgh, UK

Introduction: Inflammation is an important physiological event in the endometrium at menstruation and during early pregnancy. The lipoxins are a group of eicosanoids synthesised from arachidonic acid. They act through the G protein coupled receptor formyl peptide receptor 2 (FPR2) to have both anti-inflammatory and pro-resolution effects. The aims of this study were: (1) to assess the expression of FPR2 in the human endometrium across the menstrual cycle and in first trimester decidua and (2) to investigate the ability of lipoxins to suppress the expression of inflammatory mediators in the human endometrium and first trimester decidua.
Methods: Endometrial biopsies at different stages of the menstrual cycle (n= 34) and first trimester decidua at 6-12 weeks gestation (n=26) were obtained with Lothian Research Ethics Committee approval and informed patient consent. Tissue was serum starved overnight before treatment with 100nM phorbol 12-myristate 13-acetate (PMA) with or without 500nM lipoxin A4 for 6-8 hours. Gene expression was determined by real-time PCR and FPR2 localization by immunohistochemistry.
Results and Discussion: FPR2 mRNA expression is significantly elevated during menstruation compared with proliferative and secretory phases of the menstrual cycle ($p<0.001$). In first trimester deicuda expression of FPR2 is elevated compared with proliferative and secretory phase endometrium ($p<0.001$). FPR2 protein localises to the glandular epithelium, vascular endothelium and leukocyte populations in the stroma. Treatment of endometrial and decidua tissue with lipoxin A4 can reduce the expression of the inflammatory genes IL-6, IL-8 and COX-2 in response to PMA stimulation ($p<0.05$). These data demonstrate that lipoxin receptor (FPR2) expression is elevated at menstruation and during early pregnancy. Lipoxins can suppress the expression of inflammatory mediators in both endometrial and decidua tissue. These data highlight that lipoxins represent important anti-inflammatory modulators during menstruation and early pregnancy.

O4 The impact of ERβ dependent signalling on vascular function within the human endometrium

E Greaves[1], F Collins[1], H Critchley[2] & P Saunders[1]
[1]Human Reproductive Sciences Unit, Medical Research Council, Edinburgh, UK; [2]Division of Reproductive and Developmental Sciences, University of Edinburgh, UK

Introduction: The human endometrium is a sex steroid target tissue that undergoes cyclical remodeling. Within the endometrium angiogenesis occurs during the proliferative (oestrogen-dominated) phase of the menstrual cycle resulting in vascular bed replenishment following menses. The function of the microvasculature is organ specific, we have therefore used endothelial cell lines derived from uterine tissue for these studies. The impact of oestrogens on endometrial function is mediated by oestrogen receptors alpha (ERα) and beta (ERβ) that show different spatial and temporal patterns of expression during the normal cycle. Our immunohistochemical studies have previously revealed that endometrial endothelial cells are ERβ+/ERα-.
Methods: In the current study we used a range of molecular and functional techniques to investigate the impact of oestrogen and ERβ agonists (10-8 M) on TERT-immortalized human endometrial endothelial cells (HEEC).
Results and Discussion: HEEC expressed mRNAs encoding full length (ERβ1) and splice variant isoforms of ERβ (ERβ2, ERβ5) but had little ERα mRNA which was not translated into detectable protein. Incubation of cells with E2 and ERβ agonists did not induce expression of a 3xERE-luc reporter gene, however FRAP analysis using a YFP-ERβ construct introduced into cells using a viral vector revealed that incubation with E2 and ERβ agonists could activate the receptor (reduce mobility) in this cell type. Interestingly, ERβ-selective agonists had a negative impact on formation of vascular networks in an angiogenesis assay. In conclusion, ERβ-dependent changes in cell function occur in uterine endothelial cells in vitro but this may not be induced via direct binding to classical EREs. Future work will focus on the demonstration of ERβ binding to non-classical sites within ERβ regulated genes in addition to identification of ERβ dependent gene networks in this cell type.

This work is part of ongoing studies into the use of selective oestrogen receptor modulators (SERMs) for treatment of endometrial pathologies.

Monday 12th July 2010

Symposium II: Reproduction and development in exotic species
Main Auditorium
0900 - 1100

Speaker abstracts

Notes

S5 Reproduction and foetal development in elephants

TB Hildebrandt[1], R Hermes, I Lueders[1], J Saragusty[1] & F Goeritz[1]
Leibniz Institute for Zoo and Wildlife Research, D-10315 Berlin, Germany

Among our living terrestrial vertebrates, the three elephant species (*Elephas maximus, Loxodonta africana, Loxodonta cyclotis*) hold a unique position and differ in many ways from the general mammalian model. A fact that is most striking is in regard to their reproductive physiology. Many special reproductive features make the Asian and both African elephants subjects of great scientific interest. However, due to low numbers of available study animals, the limited accessibility, their enormous size and their endangered status, elephants are extremely challenging to study. Through a combination of different approaches, such as post-mortem structure analysis, biochemical investigations, the application of imaging techniques and behavioral observations, considerable advances have been made over the last four decades. This has assisted with the markedly improved breeding success of captive Asian and African elephants, as well as the management of their wild populations. The most recent accomplishments include successful manual semen collection, sorting and freezing as well as artificial inseminations with fresh, chilled or frozen semen. Elephants, with an average gestation of 20 to 23 months, have the longest pregnancy of all mammals. For this reason, their fetal development has always been of special interest. A closely related question is the determination of fetal age, since major events in prenatal development can only be evaluated if linked to gestational age. The extraordinary size of the animals and the exceptional female anatomy hampered the observation of pregnancies with conventional methods in the past. The continuous research efforts and the enhanced understanding of elephant reproductive physiology represent a key factor for in- and ex-situ conservation of these magnificent creatures in the future.

S6 From wild to domesticated in 30 years: lessons from the reproductive management of farmed red deer (Cervus elaphus)

GW Asher
AgResearch Ltd, Invermay Agricultural Centre, Private Bag 50034, Mosgiel, New Zealand

Reproductive physiology of red deer, a recent domesticant, has generally been modelled conceptually on other seasonally-breeding domestic ruminants such as sheep. While numerous studies over the last 30 years of deer farming have demonstrated the broad alignment to the accepted ovine endocrine template for events such as ovulation, pregnancy establishment/maintenance, parturition, spermatogenesis and general reproductive behaviours, it is my contention that 'the devil is in the detail'. In other words, the subtleties of how cervids, and any other non-traditional farmed species for that matter, express particular reproductive outcomes in relation to endocrine signals differ in many ways from 'model' ruminants. It is these subtleties that often foil our repeated attempts to manipulate and optimise reproductive outputs within the farmed or *ex situ* environment. In this paper I will discuss various aspects of reproductive management of farmed red deer for which there have been unexpected outcomes, such as for attempts to manipulate birthing season, to increase pregnancy rates of pubertal hinds and to adapt artificial reproductive technologies for genetic management. My central thesis is that the red deer is a species superbly adapted to highly seasonal annual reproductive cycles for survival in regions of climatic extremes and exhibit remarkably high levels of reproductive success within these environments. As such, they have evolved complex physiological buffering mechanisms to ensure adherence to this pattern in the face of short-term vagaries, and unpredictable outcomes, of climate and feed supply. Examples of this include profound environmental (non-genetic) control over gestation length, the ability to cue body-mass thresholds for puberty attainment from early-life nutritional environments and the interactive effects of various environmental modifiers of conception date (a lesson in reductionism vs. holism). These examples beg the question….do traditional domestic ruminants possess similar buffering mechanisms or has the process of domestication dampened their effect?

Notes

S7 In vitro fertilization and embryo transfer in rare felid populations

WF Swanson
Center for Conservation and Research of Endangered Wildlife, Cincinnati Zoo and Botanical Garden, Cincinnati OH, USA

In vitro fertilization and embryo transfer are potentially valuable tools for managing and conserving rare populations of endangered nondomestic cats and genetically-unique domestic cat research models. Application of these assisted reproductive technologies to divergent cat models and species requires extensive initial research in domestic cats followed by extrapolation to different felid populations and further refinement to improve technique efficiency. In our laboratory, our objectives have been to 1) assess cat embryo developmental requirements to formulate a feline-specific culture medium, 2) evaluate novel recipient synchronization methods for improved embryo transfer success, and 3) investigate comparative efficiency in 5 nondomestic felid species and 24 domestic cat models of hereditary disease. In a series of factorial experiments, ~1500 domestic cat IVF embryos were cultured in varying media formulations and evaluated for metabolism and development in vitro compared to embryos grown in vivo. In a feline optimized culture medium (FOCM), 70% of IVF embryos developed to blastocysts within an in vivo time frame. For recipient synchronization, two novel hormonal regimens – eCG/GnRH and eCG/pLH – were assessed for follicular stimulation, ovulatory response and embryo transfer. The eCG/pLH regimen reduced post-ovulatory ovarian stimulation and resulted in >80% pregnancy success and ~60% embryo survival in pregnant females. Extrapolation of findings to wild cat species (ocelot, sand cat, Pallas' cat, fishing cat, black-footed cat) resulted in improved fertilization and embryo development in FOCM compared to a standard culture medium. Following embryo transfer into pLH-treated recipients, three litters of ocelots and one litter of sand cats have been born. Viable offspring also have been produced using IVF and embryo transfer in eight domestic cat disease models. Our results suggest that most felid species and disease models have similar fertilization and embryo developmental requirements in vitro but that recipient synchronization and embryo transfer success may exhibit greater inter-species or inter-model variability.

S8 Assisted reproductive technologies as applied to canids

WK Farstad
Department of Production Animal Clinical Sciences, Norwegian School of Veterinary Science, Oslo, Norway

Introduction: Canids are a heterogeneous family of species showing a worldwide distribution of habitats from the arctic permafrost to the equatorial desert, from coastland to mountains; a majority of them seasonally oestrous, but sharing the unique features of having only one oestrus per season and ovulating metaphase I immature oocytes, with the domestic dog. Hence, the dog is a convenient model for developing assisted reproductive technologies that may be applicable to wild canids.
Methods: Several canid species around the world are threatened by inbreeding and by human intervention. In addition to protecting the animals from poaching, some from mixing with domestic dogs, and securing their habitats, artificial breeding techniques may be one of several solutions to exchange genetic material between wild or captive populations. Manipulation of the oestrus cycle, cryopreservation of semen, artificial insemination, freezing of oocytes, or whole ovaries to salvage gametes, or somatic cells that may be used for nuclear transfer with subsequent in vitro fertilisation and transfer of cultured embryos, are methods that are either established or may be applied.
Results and Discussion: In the author's laboratory we have experience with some of these procedures in dogs, farmed foxes, wolves, African wild dogs and are currently planning ways to apply some of these procedures to the Ethiopian wolf. Some of the aforementioned techniques still have limited success even in the dog. In wild canids the scarce knowledge of the reproductive cycle of some of the species, the availability of bottleneck assisted reproductive techniques, such as IVF and embryo culture, and the degree of human intervention tolerated by the wild species, represent further challenges.

Monday 12th July 2010

Oral Session 1: Female/male reproduction
Main Auditorium
1130 - 1300

Presenter abstracts

Notes

O5 Ovine fetal testis development is perturbed by in-utero exposure to a cocktail of chemicals contained in sewage sludge

RG Lea[1], SM Rhind[2], B Egbowon[1], MR Amezaga[3], C Cotinot[4], B Loup[4], B Mandon-Pepin[4], PA Fowler[3] & KD Sinclair[1]

[1]Schools of Veterinary Medicine and Science and Biosciences, University of Nottingham, Loughborough, UK; [2]Macaulay Land Use Research Institute, Aberdeen, UK; [3]Institute of Medical Sciences, University of Aberdeen, Aberdeen, UK; [4]INRA, Jouy-en-Josas, France

Introduction: Exposure to environmental endocrine disrupting chemicals (EDCs) during pregnancy has been associated with reproductive disorders in animals and humans. Fetuses from pregnant ewes exposed to EDCs in sewage sludge fertiliser (a "real life" exposure model) have fewer Sertoli and Leydig cells in the testis. The aim of this study was to assess the effects of sewage sludge exposure during key developmental periods of the fetal testis.
Methods: Pregnant ewes were exposed to sewage sludge fertilised pastures from 0–80, 30-110, 60-140 and 0-140 days of gestation. Never-exposed pregnant ewes served as controls and fetal tissues were collected post mortem at day 140 of gestation (term = day 145). Additionally, pregnant ewes exposed from 0-80 days (vs. controls) were euthanized at day 80. Fetal testes were Bouins-fixed and subjected to immunohistochemistry for AMH (Sertoli cell marker) and steroidogenic enzymes (P450scc, P450c17: Leydig cell markers). Fetal plasma testosterone was measured.
Results and Discussion: By day 80 of gestation, numbers of both AMH and P450c17 +ve cells were significantly reduced (P=0.007, P=0.023 respectively) following sludge exposure, but there was no effect on P450scc +ve cell numbers. By day 140 of gestation, both P450scc and P450c17 +ve cell numbers were reduced (P<0.005) in the day 0-140 and 0-80 treatment groups. However, only P450c17 +ve cells were (P<0.001) reduced in the day 30-110 treatment group. This may indicate the detection of different subsets of Leydig cells. At day 140, Sertoli cell numbers were only affected in the intermediate exposure groups (0–80, 30-110, 60-140) but not in animals exposed throughout; testosterone levels were reduced in the 60–140 group only (P=0.014). These data indicate that the EDCs found in sewage sludge impact on fetal testis development differently, depending on the period of gestation during which exposure occurs.

[EC (FP7/2007-2013) grant agreement 212885].

O6 The role of fibroblast growth factor 2 in regulating angiogenesis during the bovine follicular-luteal transition

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Angiogenesis is critical during the follicular-luteal transition for corpus luteum (CL) function. Fibroblast growth factor 2 (FGF2), acting through the FGF receptor (FGFR) is a potent pro-angiogenic factor. Follicular FGF2 dynamically increases after the LH surge in the cow, however its role(s) in regulating angiogenesis during this transition is unknown. Therefore, the aim of this study was to determine the role of FGF2 in endothelial cell network formation and its effect on progesterone production. Granulosa and theca cells were harvested from large bovine follicles (>10mm) and plated at 300,000 and 100,000 cells/well, respectively onto fibronectin-coated 12-well plates. In Experiment 1, cells were treated with 0, 0.1, 1, 10 or 100ng/ml FGF2 for 3 and 5 days (n=4 cultures). In Experiment 2, cells were treated with 5μM SU5402 (FGFR inhibitor) or DMSO (control) for 7 days (n=4 cultures). Spent medium was collected for progesterone analysis and at the end of culture endothelial cells were immunolocalised with von Willebrand factor (VWF). The degree of endothelial network formation was assessed by image analysis. In Experiment 1, in control cultures endothelial cells formed clusters that showed little to no sign of branching or elongation on day 3. FGF2 dose-dependently (P<0.001) dramatically changed the pattern of endothelial network formation with endothelial clusters developing branches and tubule-like structures. At 100ng/ml FGF2, endothelial cell networks had fully developed by day 3. A similar pattern was observed on day 5. In Experiment 2, there was extensive development of endothelial cell networks by day 7, while only a few unorganised endothelial cell clusters were observed in SU5402-treated wells. Indeed, SU5402 reduced area of VWF staining by approximately 90% (P<0.001). Interestingly, FGFR inhibition increased (P<0.005) progesterone production on all days. In conclusion, FGF2 plays a pivotal role in the formation of endothelial cell networks and tubule-like structures in the CL.

Notes

O7 The role of oxygen availability in ovarian follicle development and function in vitro

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In vivo, gaseous exchange occurs by diffusion from the perifollicular capillary network to the ovarian follicle interior. However, *in vitro* the avascular follicles rely on the transport of gases to and from their immediate environment. Insufficiencies in oxygen supply *in vitro* may occur owing to the low solubility of oxygen in solution. Our laboratory has shown that the standard 5% CO_2 in air gas phase (20% O_2) may not provide adequate oxygenation for optimal follicle growth and development. The central hypothesis here is that enhanced delivery of oxygen to follicles *in vitro* improves follicular growth and development. Preantral mouse follicles (180-220 μm in diameter) were mechanically isolated and cultured for 6 days in 20% or 40% O2 in 100 μl droplets of alpha minimal essential medium supplemented with 1 IU/ml FSH, 25 μg/ml ascorbic acid and 5% mouse serum. Cultures were conducted in standard round-bottom 96-well plates or in BD-Biosensor plates, which have an oxygen-permeable silicon matrix base in each well. Follicles were treated with glutathione (GSH; 1 mM) or sodium selenite (10 ng/ml). On alternate days, follicles were transferred to fresh medium and follicle diameter was measured. Conditioned medium was collected and stored at -80°C for oestradiol, lactate and vascular endothelial growth factor (VEGF) analysis. Follicles cultured in 40% O2 had significantly larger terminal diameters ($p<0.01$), enhanced oestradiol secretion ($p<0.01$), and reduced lactate ($p<0.01$) and VEGF ($p<0.05$) secretion in comparison to those cultured in 20% O_2. GSH and sodium selenite supplementation improved follicle growth ($p<0.01$). Culture of follicles in oxygen-permeable plates also improved growth relative to controls ($p<0.01$). From these data we conclude that oxygen availability *in vitro* is an important factor for optimal ovarian follicle growth and function.

O8 Identifying the role of calcium stores in flagellar motility

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Intracellular calcium concentration $[Ca^{2+}]_i$ has been implicated in regulation of all processes elemental for gamete fusion including acrosome reaction, flagellar beat and hyperactivated motility, chemotaxis and is an important contributor to capacitation. Previous studies have identified two separate Ca^{2+} stores in sperm with potentially distinct mechanisms of filling and mobilization. Reservoirs for Ca^{2+} these stores contribute to intracellular biochemical homeostasis and tight regulation of multiple Ca^{2+} dependant processes. The study aims to characterise the posterior calcium store to determine the relationship between store mobilization and hyperactivated motility in human sperm. The presence and location of both Ca^{2+} stores in human sperm have been identified using live single cell fluorescence imaging of Mag-Fluo-4 AM loaded cells to visualise and monitor store activity. In addition spermatozoa were permeabilized with Streptolysin-O a bacterial cytolysin causing a release of cytoplasmic dye so that both calcium stores are clearly observable, enabling visualization of Ca^{2+} store mobilization in response to treatment. The acrosome, the anterior Ca^{2+} store has been shown to localize inositol 1,4,5-Triphosphate receptors and empty Ca^{2+} contents in response to IP3R activation. Posterior calcium store identity is less clear, currently the redundant nuclear envelope or mitochondria are candidates. Preliminary data identifies the posterior store as the key regulator of motility and initiation of hyperactivation. Treatment with low doses of progesterone induce transient, oscillatory elevation of $[Ca^{2+}]_i$ in the midpiece of human sperm due to cyclic Ca^{2+} mobilization of the posterior store. Imaging of loosely tethered cells treated with 4-aminopyridine, an effective inducer of hyperactivation shows an elevation in $[Ca^{2+}]_i$ in the posterior store region. These findings support previous data generated by our group suggesting the presence of a second calcium store located at the anterior midpiece and a role for this store in hyperactivated motility.

Notes

O9 Testicular LH-stimulated testosterone production is inhibited by activation of melanocortin 3 receptors (MC_3)

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The role of MC_3 in the periphery is unclear although it appears to have some immunomodulatory effects. Recently we described immunopositive staining for MC_3 in the testes of wild type mice and reported that the testicular histology of the MC_3 null mouse was abnormal. The aims of this study were to confirm that MC_3 is expressed in testes and determine if $ACTH_{1-39}$ affects testicular steroidogenesis *in vitro*. RNA and protein were extracted from the testes of adult wild type mice (C57 B1.6) for use in RT-PCR and Western blotting, respectively. Testes were also hemi-sected and incubated ± $ACTH_{1-39}$ (10^{-16}-10^{-8}M) ± LH (2 ng/ml) for 5 hours at 35°C in air. The media were removed and frozen until assayed for testosterone by RIA. Treatments were done in quadruplicate and each experiment was repeated 2 to 4 times. A single band of the expected PCR product size, 820 bp, was obtained: this band was absent in the negative control. A band, as expected at 40 kDa, was detected by Western blotting. Testes incubated in the absence of LH, released 150±40 pg testosterone/mg of tissue (mean±SEM; n=16) into the media over 5 hrs and this was not modified by the addition of $ACTH_{1-39}$ regardless of the concentration used. Addition of LH significantly increased the amount of testosterone released into the media (mean±SEM = 620±80 pg/mg of tissue, n=8: $P<0.05$) whilst the addition of $ACTH_{1-39}$ resulted in the inhibition of steroid production (55% inhibition in the presence of 10^{-12}M $ACTH_{1-39}$: $P<0.05$). Both mRNA and protein for MC_3 have been detected in adult mouse testes. $ACTH_{1-39}$ has no effect on the basal production of testosterone but appears to inhibit LH-stimulated production. MC_3 signalling may have a modulatory role in the male reproductive axis: further work is required. *Funded by the SfE, Nuffield Foundation and SRF.*

Funded by SRF Vacation Scholarship

O10 A putative role for prokineticins in infection-induced preterm labour - an amnion perspective

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Introduction: Preterm labour is associated with premature activation of pro-inflammatory and contractile cascades and can be a consequence of infection. Given the well described roles for prokineticins (PROK) in induction of inflammation and smooth muscle contractility, we hypothesized that PROK signaling is a component of infection-induced preterm labour. We investigated (a) the expression of PROK1 and PROK1 receptor (PROKR1) and (b) effects of a mimetic of infection on *ex vivo* expression of PROK1 and PROKR1 in human amnion and mouse fetal membranes.
Methods: Amnion was obtained after informed consent with ethical approval from women undergoing elective caesarean deliveries at term (defined as >37 weeks gestation). Mouse fetal membranes were collected on D16-19 of gestation. Tissues were fixed (for immunohistochemistry), cultured *ex vivo* with a mimetic of infection (100ng/µl lipopolysaccharide (LPS)), or stored in RNAlater for subsequent qPCR analysis. PROK1, PROKR1, cyclo-oxygenase (COX)-2, interleukin (IL)-8 and IL-6 expression was assessed by qPCR and localization of PROK1 and PROKR1 was assessed by immunohistochemistry.
Results and Discussion: PROK1 and PROKR1 were immunolocalised to epithelial cells of human amnion and mouse fetal membranes. Treatment of human amnion with LPS increased expression of PROKR1 ($p<0.01$). In mouse fetal membranes, endogenous expression of PROK1 was highest on D18 of pregnancy ($p<0.01$) and *ex vivo* treatment with LPS on D17 increased expression of PROK1 ($p<0.01$). In addition, treatment with LPS increased expression of inflammatory mediators in human amnion and mouse fetal membranes (such as COX-2, IL-6 and IL-8; $p<0.01$). These data highlight a role for PROK1 in fetal membranes and suggest that mouse fetal membranes may be a suitable model for the study of infection-induced preterm labour. Elevated expression of PROK1 in fetal membranes shortly prior to onset of labour (D18 of pregnancy) suggests a putative role for this cytokine in initiation of fetal membrane rupture.

Monday 12th July 2010

Oral Session 2: Ovary/uterus
Lecture Block A02
1130 - 1300

Presenter abstracts

Notes

O11 The consequences of germ cell loss on ovarian development in neonatal Dazl knock-out mice

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During fetal development in mice, primordial germ cells migrate into the genital ridge, proliferate then enter meiosis. Somatic cells surround each germ cell and, around birth, become primordial follicles. When the germ cell specific protein, Dazl is absent in Dazl knock out (ko) mice, all oocytes are lost by pnd7. However, the remaining ovarian tissue becomes steroidogenically active and hypertrophies to form tumors as mice age. The present study determined the immediate effect of oocyte loss on the remaining ovarian tissue from ed18 to pnd21 in Dazl ko mice compared with wt and het sibling ovaries as controls. Using immunohistohemistry, oocytes were identified by MVH, development of granulosa cells by AMH, inhibin/activin and follistatin, steroidogenic competence by 3β and 17αHSD expression and surface epithelium (OSE) and sex cords by cytokeratin and laminin staining. Morphologically, there was no difference between wt/het and ko ovaries at ed18. However, by pnd0 in ko ovaries the few MVH positive germ cells present were apoptotic. On pnd1 these ovaries lacking germ cells were composed of granulosa-type cells expressing AMH, inhibin α and activin βA and βB subunits and by pnd7 were also expressing 3βHSD with few fibroblast type cells and had a highly proliferative surface epithelium. Follistatin expression in ko ovaries from ed18 onwards would indicate activin modulation despite lack of oocytes. All genotypes expressed high levels of cytokeratin in the OSE but this was much more active in d21ko than wt/het ovaries. In conclusion, oocyte depleted ovaries were composed of granulosa-type cells, epithelium, and few fibroblast type cells which form the basis of all hypertrophied ovaries in adults. Activation of granulosa cell markers appeared to be pre-programmed as their temporal expression was similar in all genotypes regardless of the presence of oocytes, while OSE proliferation may be regulated by oocytes.

O12 Toll-like receptor 4 (TLR4) mediates the immune response of epithelial and stromal cells to bacterial lipopolysaccharide in the endometrium associated with pelvic inflammatory disease

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Introduction: Bacterial infections of the female genital tract cause pelvic inflammatory disease and preterm labour in women. Lipopolysaccharide (LPS) is a potent pathogen-associated molecule that induces inflammation during bacterial infections. Innate immunity depends on the binding of LPS to Toll-like receptor 4 (TLR4) on mammalian cells. This leads to secretion of pro-inflammatory cytokines, prostaglandins, and chemokines that attract neutrophils and macrophages to the site of infection. The present study tested the hypothesis that TLR4 mediates the immune response to LPS in the endometrium associated with pelvic inflammatory disease.
Methods: Wild type (WT) and TLR4-deficient ($TLR4^{-/-}$) mice were infused intrauterine with ultra-purified LPS *in vivo* and pelvic inflammatory disease evaluated by histology; study approved by UK Home Office. Endometrial epithelial and stromal cells isolated from mice and women were challenged with LPS *in vitro*; endometrial cells were also co-cultured with macrophages. The cytokine IL-6, prostaglandin E2 (PGE), and chemokines CXCL1 and CXCL8, were measured by ELISA.
Results and Discussion: Infusion of LPS caused pelvic inflammatory disease in WT mice, with influx of neutrophils and macrophages into the endometrium. The $TLR4^{-/-}$ mice infused with LPS, or either genotype infused with vehicle, did not develop pelvic inflammatory disease. Bacterial LPS stimulated secretion of IL-6, PGE and CXCL1 in a concentration and time-dependent manner, by endometrial epithelial or stromal cells from WT but not $TLR4^{-/-}$ mice. However, $TLR4^{-/-}$ endometrial cells did respond to pathogen-associated molecules acting through innate immune receptors other than TLR4. Co-culture of $TLR4^{-/-}$ endometrial cells with WT endometrial cells or macrophages only proportionately rescued the IL-6, PGE or CXCL1 response to LPS. Human endometrial stromal cells secreted IL-6 and CXCL8 in response to LPS, in a concentration and time-dependent manner. In conclusion, TLR4 on epithelial and stromal cells of the endometrium mediates the immune response to LPS associated with pelvic inflammatory disease.
Studies approved by Home Office and Local Ethical Review Panels or Committees.
(Funded by BBSRC BB/D02028X).

Notes

O13 Exposure to a cocktail of environmental chemicals at critical windows of fetal development: does timing matter to the fetus?

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Introduction: It is well documented that the precise timings of acute exposure to single environmental chemicals (ECs) during gestation influence health outcomes. We have previously shown that in-utero exposure to complex mixtures of ECs in sewage sludge fertiliser disrupts the fetal ovine ovary. We aimed to assess the effects of sewage sludge exposure at critical stages during ovine ovarian ontogeny.
Methods: Pregnant ewes were exposed (T) to pastures fertilised with sewage sludge from 0-80, 30-110, 60-140 or 0-140 days of gestation. Control ewes (0-140C) were pastured on fields fertilised with inorganic fertiliser. Ovaries were collected for proteomic analysis on day 140 of gestation; 2-DE profiles were compared with SameSpots software and proteins identified using LC/MS/MS.
Results and Discussion: Exposure group 60-140T had reduced uterine weight ($p<0.05$) and greater anogenital distance (AGD) than any other group ($p<0.05$), except 30-110T. Testosterone was significantly increased in 0-80T ($p<0.05$) but not in groups with high AGD, suggesting that mechanisms other than elevated testosterone were responsible. Free T3 was significantly reduced in 0-140T ($p<0.05$). 73/776 protein spots were differentially expressed ($p<0.05$) and 25 were identified. Proteomes of 30-110T and 60-140T were more affected than other treatment groups. Proteins and pathways altered included: i) detoxification/immunity (GSTM1, LTF, HMGB1); ii) metabolism (ALDH1A2, HDHD2, ACSF2, PHGDH, TF); iii) cell cycle, transcription and signalling (HNRNPK, PSPC1, HMGB1, COPS4, HSPA9); iv) structure and development (lamin, GSN, TAGLN, DPYSL2, PHGDH). 2.2-fold increased expression of PSPC1 (coregulator of androgen receptor-mediated transcription) in 60-140T suggests alterations in androgen-sensitivity. Our data indicate that the effects of in-utero exposure to complex mixtures of ECs differ depending on the timing of exposure. Late gestational exposure caused more alterations in the fetus than early exposure. This suggests that the health risks to the fetus subjected to low levels of EC mixtures will vary depending on the stage of gestation.
(Funded by FP7-212885).

O14 Interactions between Prolactin and Estrogenic Hormones in Regulating Uterine Glycogen Metabolism in Mink *(Neovison vison)*

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In mink, hormonal regulation of uterine and embryonic development before implantation involves interactions between many hormones including estradiol 17-beta (E2) and prolactin (PRL). During that time, the uterus sequesters glucose as glycogen (GLY), a uterine histotroph nutrient. We have shown that E2 and 4-hydroxyestradiol (4-OHE2), a catechol-metabolite of E2, increase uterine GLY reserves in mink. Our objectives were to determine: 1. If PRL influences uterine GLY metabolism, 2. If the uterus expresses genes for PRL, PRL receptor (PRL-R), E2 receptor alpha (E2-R alpha), and CYP1b1, the enzyme that catalyzes 4-OHE2 synthesis, and 3. if PRL and/or E2 influence expression of those genes. Mink were ovariectomized and treated with E2, haloperidol (HAL, a dopaminergic antagonist to increase PRL secretion), HAL + E2, or as controls (approved by the Institutional Animal Care and Use Committee). Gene expression levels were determined by qPCR. GLY levels were determined by measuring glucose concentrations spectrophotometrically, before and after GLY hydrolysis. We discovered that: 1. PRL had no effect on total uterine GLY concentrations, 2. mink uteri expressed mRNAs for PRL, PRLR, E2R-alpha, and CYP1b1, 3. PRL increased CYP1b1, PRL and PRL-R expression, and 4. E2 increased PRL-R, E2R-alpha and CYP1b1 expression. We propose that during the follicular phase, E2 promotes GLY synthesis by increasing tissue responsiveness to PRL and E2, resulting in increased CYP1b1 expression, and subsequent 4-OHE2 production. As circulating PRL concentrations increase throughout pregnancy, this hormone may stimulate uterine PRL and PRL-R expression, also elevating CYP1b1 expression and 4-OHE2 production. The amplifying effects of E2 and PRL on uterine 4-OHE2 production may serve to ensure adequate uterine GLY synthesis prior to ovulation and during the uterine secretory phase, supporting embryonic growth and implantation.
[Funded by Fur Commission USA and NIH INBRE, P20RR016454].

Notes

O15 Vectorial secretion of prostaglandins by epithelial cells from the bovine endometrium

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Introduction: In cattle, endometrial epithelial cells secrete prostaglandin $F_{2\alpha}$ (PGF) and E2 (PGE). Prostaglandins have important roles in the regulation of ovarian cycles and in the response to pathogens such as Escherichia coli and it's lipopolysaccharide (LPS). In the endometrium, stromal-epithelial cell interactions and the formation of tight junctions by polarised epithelial cells are critical for physiological function. However, '2D' monoculture of epithelial cells *in vitro* does not reflect the normal organisation of the endometrium. Here, we tested the hypothesis that polarised epithelial cells secrete prostaglandins in a vectorial manner under physiological and pathological conditions.
Methods: Epithelial cells isolated from bovine endometrium (n=3) were cultured as polarised layers on hanging inserts in 24-well plates; some inserts were co-cultured with stromal cells seeded in the well below. Cells were treated for 24 h with control media; 100 nM oxytocin and 100 µM arachidonic acid (OT+AA); or 1 µg/ml E. coli LPS. Transepithelial resistance was measured to monitor epithelial cell monolayer integrity. Accumulation of PGF and PGE in the apical and basolateral supernatants was measured by radioimmunoassay.
Results and Discussion: Confluent polarised epithelial cells had a consistent transepithelial resistance of >2000 Ωcm^2, and this was not affected by treatment. Prostaglandin accumulation was stimulated in epithelial cells treated with OT+AA or LPS. However, the accumulation of PGF was greater in the basolateral than the apical compartment with OT+AA (325±10 vs. 64±15 ng, $P<0.05$) or LPS (62±32 vs. 3±1 ng, $P<0.05$). This was similar for PGE (OT+AA: 363±21 vs. 114±15 ng, $P<0.05$; LPS: 40±11 vs. 13±4 ng, $P<0.05$). Co-culture with stromal cells did not significantly modulate prostaglandin secretion of treated cells. In conclusion, epithelial cells preferentially secrete prostaglandins basolaterally under physiological and pathological conditions; this may maximise the transport of prostaglandins into the venous circulation to exert downstream effects. (Work funded by BBSRC).

O16 The effects of a low therapeutic dose of βCG fragment-lytic peptide conjugates on gonadotropin secretion and ovarian function in ewes

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Introduction: Conjugated lytic peptides consist of small, cell-disrupting peptides attached to the fragments of gonadotropic hormones, allowing them to target cancer tissue expressing the LH/hCG receptor. The aim of the present study was to examine the effects of two lytic peptide conjugates, Phor21-βCG(ala) and βCG(ala)-Phor21, at a low therapeutic dose (0.2 mg/kg b.w.), on periovulatory ovarian activity and ensuing luteal function in sheep. We hypothesized that the dense expression of lutropin receptors on the preovulatory follicle would present an appropriate target for the drugs and disrupt normal ovarian dynamics.
Methods: Serum levels of reproductive hormones and ultrasonographic images were used for the assessment of periovulatory events following drug administration in 14 ewes; seven animals served as untreated controls. Estrus and ovulations were synchronized with intravaginal progestin-releasing sponges that were left in place for 12 days and a single i.m. injection of 750 IU of eCG given at sponge withdrawal. Both drugs were administered by an i.v. injection 36 h post-sponge removal, at the expected onset of the preovulatory surge of gonadotropins.
Results and Discussion: No difference ($P>0.05$) was detected in the number of luteal structures per ewe in control versus treated animals during early luteogenesis. After drug administration, circulating E_2-17β concentrations were lower ($P<0.05$) in βCG-Phor21(ala)-treated animals and mean serum concentrations of progesterone were lower ($P<0.05$) in βCG-Phor21(ala)-treated than control animals. There were no differences ($P>0.05$) in the percentage of ewes that lambed or lamb characteristics between the 3 groups at lambing 9 months after the treatment. In summary, neither Phor21-βCG(ala) nor βCG(ala)-Phor21 demonstrated adverse effects on the ovulatory process and formation of luteal structures but the treatment with βCG(ala)-Phor21 significantly depressed ovarian steroidogenesis. With a lack of evidence for disruptive effects on ovulation, endocrine function and post-treatment fertility, these results support the use of Phor21-βCG(ala) as a cancer pharmaceutical.

This study was funded by the Natural Sciences and Engineering Research Council (NSERC) of Canada (PMB).

Monday 12th July 2010

Oral Session 3: Oocyte/embryo
Lecture Block A03
1130 - 1300

Presenter abstracts

Notes

O17 Knockdown of p27^{Kip1} induces the growth of oocytes in mice

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Introduction: In mice, small oocytes are enclosed within flattened granulosa cells to form primordial follicles around birth. A small number of these oocytes in primordial follicles (primordial oocytes) start to grow, whereas others remain quiescent. The mechanism regulating the growth of primordial oocytes are not well understood. The objective of this study is to know the role of p27^{Kip1}, known to regulate cell cycle progression in somatic cells, in the growth of primordial oocytes in neonatal mice.
Methods: We studied the localization of p27^{Kip1} in 0-, 3-, 5-, 7- and 21-day-old mouse ovaries by immunohistochemistry. Ovaries from 3- to 5-day-old mice were treated with p27^{Kip1} siRNAs and knockdown of p27^{Kip1} was determined by immunohistochemistry and Western blotting. siRNA-treated ovaries were cultured for 6 days in α-MEM supplemented with 5% fetal calf serum and the oocyte growth was examined histologically. This study was approved by the Institutional Animal Care and Use Committee and carried out according to the Guidelines of Animal Experimentation of Kobe University, Japan.
Results and Discussion: p27^{Kip1} was expressed in nuclei of a few primordial oocytes (2 ± 2%; n=3) in newborn mice. The percentage of p27^{Kip1} positive oocytes increased to 21 ± 7(n=3), 57 ± 8 (n=3) and 66 ± 9 (n=3) in 3-, 5- and 7-day-old mice, respectively. After knockdown of the p27^{Kip1} protein by siRNAs, the proportion of growing oocytes (>40 □m in diameter) increased (5 ± 1%; n=3) in *in vitro*-cultured ovaries than that in the control (1 ± 0%; n=3). These results suggest that p27^{Kip1} negatively regulates the growth of primordial oocytes and knockdown of p27^{Kip1} enhances oocyte growth in mice.

O18 Deletion of the BH3-only gene puma protects oocytes from apoptosis and preserves fertility following anti-cancer therapy-induced DNA damage

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Anti-cancer therapy-induced DNA damage can cause primordial follicle oocyte depletion resulting in female sterility. p63, the predominant p53 family member expressed in primordial follicle oocytes, is essential for death following DNA damage. p53 exerts pro-apoptotic effects in part through transcriptional induction of the BH3-only Bcl-2 family members, Puma and Noxa. We used gene-targeted ('knock-out') mice to investigate the hypothesis that Puma and Noxa are critical down-stream apoptosis effectors for p63 following DNA damage in primordial follicle oocytes. Postnatal day (PN) 5 puma-/-, noxa -/-, puma-/-noxa-/-, or wild type (wt) mice (n=4-8/genotype) were exposed to γ-irradiation (0.45 Gy) and ovaries were harvested at PN10 for follicle enumeration. γ-irradiation completely destroyed the primordial follicle pool in wt mice, whereas 16% ± 3% of primordial follicles were protected from γ-irradiation-induced apoptosis in mice lacking Puma, and 52% ± 6% were rescued from apoptosis in mice lacking both Puma and Noxa ($p<0.001$ vs. wt). To test fertility, Puma-/-mice were irradiated (0.45 Gy) at PN5 and breeding studies commenced 45 days later. Remarkably, 13/16 γ-irradiated puma-/- females produced healthy offspring when mated with non-irradiated wt males, whereas all (5/5) γ-irradiated wt females were infertile. We conclude that puma-/- primordial follicle oocytes rescued from γ-irradiation induced apoptosis are functionally robust and capable of giving rise to normal healthy offspring. Inhibition of p63-mediated apoptosis in primordial follicle oocytes, through blockade of Puma, has implications for the design of therapeutics for the prevention of infertility following anti-cancer treatment.
This work was supported by the NHMRC of Australia (Program Grants #494802 and #257502, Fellowships JKF (#441101), KJH (#494836), CLS (#406675), AS (#461299); the Cancer Council Victoria (EMM and EN); the Leukemia and Lymphoma Society (New York; SCOR grant #7015), the National Cancer Institute (NIH, US; CA 80188 and CA 43540); Victorian Government's Operational Infrastructure Support Program.

Notes

O19 Mechanism of de-novo centriole formation in mouse embryos

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Introduction: The centrosome consists of a pair of perpendicular centrioles surrounded by the pericentriolar matrix (PCM). In interphase centrosomes are responsible for the nucleation of microtubules and organise the spindle poles in mitosis. Normally, the centrosome replicates once and only once per cell cycle in order to regulate centrosome number. Interestingly, somatic cells possess the capacity to generate replacement centrioles 'de-novo' if the resident centrioles are artificially removed. Early mouse embryos lack centrioles, but manufacture them at blastocyst stage, and thus present a rare example of de-novo centriole synthesis in a physiological setting. However, how this process is regulated is unknown.
Methods: MF1 female mice were mated with GFP-centrin2 males and embryos cultured to blastocyst stage. Morpholinos and mRNAs were microinjected and oocytes/embryos imaged using confocal microscopy.
Results and discussion: Using mice expressing GFP-centrin2, we show that centrioles emerge de-novo in blastocysts. We also find that the centriole replication proteins SAS-6 and cep192 are readily detectable by RT-PCR in blastocysts, and that the structural procentriole component SAS-6, is immunolocalised to centrosomes in blastocysts, but not morulae, suggestive of involvement in centriole formation. Overexpression of GFP-SAS-6 in normally acentriolar 2-cell embryos induced cytoplasmic accumulations which are capable of organising the PCM and microtubules, and can organise spindle poles, implying centriole-like function. However, no accumulations form in oocytes, suggesting increased hostility of the ooplasm to the presence of new centrioles. Finally we show that a prolonged S-phase arrest can induce the precocious formation of centriole-like structures in normally acentriolar 2-cell embryos, implying all the machinery necessary for centriole manufacture is already present at the 2-cell stage. We conclude that de-novo centriole formation and classical centriole replication share some of the same mechanisms, and hypothesise that an as-yet unidentified factor restrains centriole manufacture in oocytes and early embryos.
Supported by the MRC.

O20 Epigenetic reprogramming of the porcine germline

SMW Hyldig[1], N Croxall[2], DA Contreras[2], G Valdez[2], PD Thomsen[1] & R Alberio[2]
[1]Department of Basic Animal and Veterinary Sciences, Faculty of Life Sciences, University of Copenhagen, Frederiksberg C, Denmark; [2]Division of Animal Sciences, School of Biosciences, University of Nottingham, Loughborough, UK

Introduction: Genome wide DNA demethylation is critical for genome regulation during the development of the germline. Erasure of gender specific imprints and partial demethylation of repetitive elements occurs in germ cells colonizing the mouse gonad. In this study we investigated the erasure of DNA methylation marks of specific sequences in the germline of the domestic pig.
Materials and Methods: Porcine primordial germ cells (PGCs) were isolated by FACS on the basis of their OCT3/4 expression from embryos between E22 and E31 post insemination. Methylation of regulatory elements of the imprinted genes IGF2/H19 and IGF2R, and short and long interspersed nuclear elements (SINE and L1) was investigated using bisulfite sequencing analysis. Furthermore, cell cycle stage was established in PGCs.
Results and Conclusions: Demethylation of IGF2/H19 was initiated between E22 (67.6%) and E25 (27.3%) in females and reached 18.3% by E31. The DMR2 of the IGF2R was highly demethylated in males by E22 (14%), however in females demethylation was delayed until E31 (2.4%). SINE and L1 showed high methylation at E22 and E25 (55-66%) and demethylation was evident in SINE by E29-31 (~30%). Cell cycle analysis of gonadal PGCs (E22-31) showed a normal pattern of cycling cells, however, PGCs collected from an earlier stage (E17) showed increased proportion of cells in G2, suggesting that cell cycle arrest might occur in migratory PGCs in the pig. Our study shows DNA methylation reprogramming in pig gonadal PGCs. DNA repeats undergo partial demethylation, whereas imprinted genes show asynchronous DNA demethylation for different loci between E22-E31.

Notes

O21 Measurement of ATP levels and ATP consumption in the maturing oocyte

CM Dalton[1], G Szabadkai[1], H Imamura[2,3], H Noji[2], MR Duchen[1] & J Carroll[1]
[1]Department of Cell and Developmental Biology, UCL, London, UK; [2]Institute of Scientific and Industrial Research, Osaka University, Osaka, Japan; [3]Precursory Research for Embryonic Science, Japan Science and Technology Agency, Tokyo, Japan

Introduction: Mitochondria are the primary source of ATP for the maturing oocyte. Defects in both stage-specific re-distributions of mitochondria, and mitochondrial function during meiosis have been linked to decreased fertility and increased incidence of aneuploidy, however the exact relationship between cellular ATP levels and these pathologies has not yet been established. Here we used a novel FRET-based recombinant ATP probe to examine ATP levels and the rates of ATP consumption at different stages of oocyte maturation, and their relationship to cell-cycle regulated events. In addition, we inhibited mitochondrial function to examine the effect on the first meiotic spindle.
Methods: Germinal vesicle stage oocytes were collected from MF1mice 48 hours after intra-peritoneal injection of PMSG. For MII oocytes, hCG was administered 48 hours after PMSG and oocytes collected 12 hours later. Oocytes were microinjected with MAP7-EGFP mRNA or mRNA for an ATP FRET-based indicator (ATeam) for observation of spindle dynamics and measurement of ATP levels respectively. After allowing 2 hours for mRNA expression, oocytes were imaged using a confocal microscope.
Results and Discussion: ATP levels in oocytes at GV, MI and MII were similar but we observed striking differences in ATP consumption, with maturing oocytes and those arrested at MII displaying significantly higher levels of ATP consumption than those arrested at GV stage. This suggests a tight regulation of ATP supply and demand, with production being upregulated to match an increasing requirement in the maturing oocyte. Mitochondrial function appears to be essential to the maintenance of the first meiotic spindle since inhibition of mitochondria with either oligomycin or FCCP results in rapid collapse of the spindle. These results are in line with the notion that mitochondrial function is essential to maintain fertility and avoid aneuploidy, and shed light on the patterns of ATP regulation in the maturing oocyte.

O22 Involvement of selected kinases in cytoplasmic polyadenylation element binding protein dependent translational regulation during maturation of porcine oocytes

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Introduction: Regulation of translation by cytoplasmic polyadenylation of specific mRNAs is known to be important for oocyte maturation and further development. This process is mainly driven by phosphorylation of cytoplasmic polyadenylation element binding protein (CPEB). The aim of this study is to determine kinases involved in CPEB phosphorylation and consequently the CPEB-dependent polyadenylation during mammalian oocyte cell cycle.
Methods: We cultivated porcine oocytes in the presence of specific kinase inhibitors (roscovitine, KN-93 and ZM447439). We employed the poly(A)-test PCR to monitor the effect of inhibitors on poly(A)-tail extension of cyclin B1 and mos mRNAs as the markers of CPEB-dependent cytoplasmic polyadenylation. In addition, the effect of inhibitors on CPEB phosphorylation was observed by 2D–PAGE western blot.
Results and Discussion: Our results show that CPEB is modified already in the GV-stage porcine oocytes and is intensively phosphorylated at the time of GVBD. This late phosphorylation, assumed responsible for CPEB degradation in metaphase I, is sensitive to roscovitine implying it is mediated by CDK1 in porcine oocytes. Furthermore, the inhibition of CDKs from the beginning of maturation affected the cyclin B1 mRNA polyadenylation. Thus CDKs may play a role upstream the regulatory cascade leading to the CPEB-mediated polyadenylation. The CaMKII and Aurora kinase inhibitors (KN-93 and ZM447439) affected later process of maturation indicating different role of these kinases in meiotic maturation of porcine oocytes.

(Financial support from specific university research (MSMT no. 21/2010), Institutional Research Programme (IAPG No. AV OZ 50450515) and Czech Science Foundation GACR 204/09/H084.)

Monday 12th July 2010

SRF Student Prize
Main Auditorium
1430 - 1600

Presenter abstracts

Notes

O23 Neonatal exposure to aromatisable androgen or estrogen produces a reproductive and metabolic PCOS phenotype in the rat

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Approximately 20% of women have polycystic ovaries (PCO), and around 5% develop the syndrome (PCOS), associated with hyperandrogenism, infertility and metabolic consequences. Studies in sheep and rhesus monkeys suggest that PCO(S) develops as a consequence of androgen overexposure during fetal life. Previously we showed that treatment with the aromatisable androgen, testosterone proprionate (TP), during rat neonatal life (pnd 1 to15) caused a PCOS-like phenotype. Here, we compare the reproductive and metabolic effects of TP exposure with other steroids to determine which produce this PCO(S) phenotype in adults. Neonatal female rats were injected on postnatal day (pnd) 1 and 4 with either control oil, 20mg/kg TP, 10mg/kg dexamethasone (DEX), or 1mg/kg dihydrotestosterone (DHT), dehydroepiandrosterone (DHEA), or estradiol valerate (EV), and the effects of these treatments assessed at pnd60 when adult. Steroid doses were based on previous studies. TP and EV induced PCO-like ovaries with no CL which were significantly smaller ($P<0.001$ and $P<0.01$ respectively) when compared to controls. However, phenotypically normal ovaries comparable to controls were present after treatment with DHT, DHEA or DEX. Adult body weights were comparable to the control group (217.3±2.6g) in DEX and DHEA treated animals. By comparison EV treated animals were significantly heavier (261.3±5.0g, $P<0.001$) as were animals from the TP (227.6±4.1, $P<0.05$) and DHT (232.6±4.4g, $P<0.01$) groups when compared to controls. Furthermore, compared with controls, TP and EV treatment did not alter retroperitoneal fat pad weight but mesenteric fat weight was significantly increased in EV ($P<0.05$) and TP ($P<0.01$) treated animals, whereas animals treated with corticosteroid DEX showed a small but significant decrease in both retroperitoneal and mesenteric fat pad weights ($P<0.05$). These results suggest that induction of a PCO(S) phenotype in androgenised animal models is largely due to an estrogenic effect at both the reproductive and metabolic level.

O24 STIMulating sperm! STIM-Orai (store-operated) channel subunits are expressed in human sperm and may be a route for rapid Ca2+ influx

K Nash[1], L Lefievre[1,2] & S Publicover[1]
[1]School of Biosciences, The University of Birmingham, Birmingham, UK; [2]Reproductive Biology and Genetics Group, The Medical School, The University of Birmingham, Birmingham, UK

Depletion of intracellular Ca2+ stores induces extracellular Ca2+ entry through plasmalemma store operated channels (SOCs), known as store-operated calcium entry. Upon calcium-store depletion STIM, a calcium-sensing, trans-membrane protein of intracellular stores, translocates to an area adjacent to the plasma membrane and congregates to form 'puncta'. Interaction of STIM with Orai (Ca2+-channel forming) subunits activates store-operated Ca2+ entry (1). Ca2+ stores are present in human sperm (2) so we have investigated the presence and function of STIM-Orai SOCs. Sperm were harvested by direct swim-up into sEBSS (0.3% BSA) and were incubated for 5-6 hours at 6 million cell ml-1. Western blotting, immunoprecipitation and immunofluorescence (antibodies from ProSci, CA, USA) were used to identify and localise STIM and Orai subunits in human sperm. Single cell fluorescent imaging was used to assess [Ca2+]i in human sperm loaded with Oregon Green BAPTA-1. STIM isoforms were localised to the midpiece (STIM1) or more widespread (STIM2). Orai was present in the flagellum (particularly midpiece) and over the acrosome, sites of intracellular Ca2+ stores. Mobilisation of stored Ca2+ with thapsigargin (10μM) or bis-phenol (20μM) caused elevation of [Ca2+]i but no visible redistribution of STIM. STIM-Orai channels are potently activated by 5-10μM 2-aminoethoxydiphenyl borate (2-APB) but inhibited at higher concentrations (50-100μM) (1). 5μM 2-APB caused only a modest increase in [Ca2+]i but greatly potentiated the [Ca2+]i transient induced by subsequent application of 3μM progesterone. Pre-treatment with 5μM 2-APB also amplified the response to 100nM progesterone. 50-100μM 2-APB had no effect or inhibited the progesterone response. We suggest that SOCs formed from STIM-Orai subunits are a primary route for rapid Ca2+ influx in human sperm and contribute significantly to activation of sperm by progesterone. 1. Varnai, P. et al. (2009). Trends Pharm Sci 30:118-28 2. Costello et al. (2009). Reproduction 138:425-37.

Notes

O25 Transcription factor interactions during lineage segregation in the pig embryo

DA Contreras[1], SMW Hyldig[2], G Valdez[1], N Croxall[1], R Webb[1] & R Alberio[1]
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Introduction: Mammalian early embryo development is characterized by two successive cellular segregation events. In mouse embryos interaction of the transcription factors Oct-4, Cdx2, Nanog, and Gata-6 are critical determinants of these events. Oct-4 and Cdx2 expression are mutually antagonistic and demarcate the inner cell mass (ICM) and trophectoderm (TE), respectively. Similar antagonism is observed between Nanog and Gata-6, which demarcate individual cells in the ICM, and later give rise to the epiblast (Nanog expressing cells) and hypoblast (Gata-6 expressing cells). In the pig, however, Nanog is absent from the ICM, suggesting a different interaction between these molecules during lineage segregation. Here we investigated the relationship of these factors during early development in the pig.
Methods: In vivo embryos collected from pre-streak to early primitive streak stages were processed for immunohistochemistry or in situ hybridization to determine expression of these four transcription factors.
Results and Discussion: Experiments show that Oct-4 expression (RNA and protein) is restricted to the epiblast and Cdx2 is confined to TE in all analysed stages. Nanog protein is detected, as expected, exclusively in the epiblast, whereas Gata-6 in the hypoblast and, unexpectedly, within all epiblast cells in all stages. The results demonstrate that the antagonistic relationship of Oct-4-Cdx2 is maintained during TE segregation in the pig. Furthermore, the lack of mutual antagonism between Nanog and Gata-6 in the pig epiblast indicates that the relationship between these molecules differs to what is reported in mice. The co-expression of these factors also suggests that the epiblast contributes to the development of the hypoblast in the pig. In conclusion, Nanog expression in the epiblast indicates that pluripotency is established later in the pig. This supports the hypothesis that the hypoblast segregates from the epiblast, rather than from the ICM as in mice, suggesting key developmental differences between these two species.

(This project and DA Contreras were funded by Conacyt-Mexico, Royal Society, University of Nottingham).

O26 Lipoxins are anti-inflammatory modulators in endometrial adenocarcinoma

SP Rajagopal[1], SC Boddy[1], A Williams[3], RA Anderson[2], KJ Sales[1] & HN Jabbour[1]
[1]Human Reproductive Science Unit, Medical Research Council, Edinburgh, UK; [2]Reproductive and Developmental Sciences, University of Edinburgh, Edinburgh, UK; [3]Department of Pathology, Queen's Medical Research Institute, Edinburgh, UK

Introduction: Lipoxins are novel anti-inflammatory and pro-resolution eicosanoids, synthesised from arachidonic acid through the action of specific lipoxygenase (ALOX) enzymes. Lipoxins signal through a GPCR termed FPR2. This study investigated (a) the expression of ALOX enzymes (ALOX5, 12 and 15) and FPR2 receptor in endometrial adenocarcinoma and (b) the potential of lipoxin A_4 to suppress pro-inflammatory pathways in neoplastic endometrial epithelial cells.
Methods: Endometrial adenocarcinoma tissues (N=30; well, moderately and poorly differentiated) and normal human endometrium (N=10) were obtained with informed patient consent. Ishikawa cells (an endometrial epithelial cell line from a well differentiated endometrial cancer) were treated for 6-8 hours with either 100µM phorbol 12-myristate 13-acetate (PMA) or 100nM prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) alone or with 500nM lipoxin A_4. mRNA expression was determined using quantitative RT-PCR, and localisation of FPR2 was examined by immunohistochemistry.
Results and Discussion: Expression of both ALOX5 and FPR2 were elevated ($P<0.05$ and 0.001 respectively) in moderately differentiated carcinoma compared with poorly and well differentiated carcinoma and normal endometrium. However, expression of ALOX12 or ALOX15 did not differ between the various grades of cancer or normal endometrium. FPR2 was immunolocalised to neoplastic epithelial cells, the vasculature and a population of leukocytes. Finally, treatment of Ishikawa cells with PMA or $PGF_{2\alpha}$ increased the expression of the inflammatory mediators IL-6 and IL-8 ($P<0.05$). Co-treatment of the cells with PMA or $PGF_{2\alpha}$ and lipoxin A_4 reduced the expression of IL-6 and IL-8 compared to cells treated with PMA or $PGF_{2\alpha}$ alone ($P<0.05$). These data demonstrate expression of components of the lipoxin pathway in endometrial cancer. Importantly, lipoxin A_4 can reduce the expression of inflammatory mediators in neoplastic epithelial cells. Future work will investigate the functional impact of this anti-inflammatory and pro-resolution eicosanoid on endometrial cancer growth and progression.

Notes

O27 The expression and regulation of endometrial adrenomedullin (ADM): a novel candidate for post-menstrual repair

JA Maybin[1], S Battersby[2], N Hirani[3], HOD Critchley[1] & HN Jabbour[2]
[1]Centre for Reproductive Biology, University of Edinburgh, Edinburgh, UK; [2]Human Reproductive Sciences Unit, MRC, Edinburgh, UK; [3]Centre for Inflammation Research, MRC, Edinburgh, UK

Introduction: The human endometrium has a remarkable capacity for efficient repair. Factors involved remain undefined. ADM is a pluripotent peptide with a putative role in repair. During the late-secretory phase progesterone is withdrawn, stimulating prostaglandin (PG) synthesis and vasoconstriction. This results in local and transient hypoxia. We aimed to determine (i) endometrial expression of ADM throughout the menstrual cycle and (ii) its regulation by PGs and hypoxia.
Methods: Endometrial biopsies from healthy women were collected with ethical approval and consent and categorised as menstrual (n=5) proliferative (n=11), early- (n=7), mid- (n=6) and late-secretory (n=6). Endometrial explants and epithelial cells were treated with vehicle,100nM PGE_2, $PGF_{2\alpha}$ or hypoxia (0.5% O2). Gene expression was determined by Q-RT-PCR and protein by immunohistochemistry/ELISA.
Results: ADM mRNA expression peaked at menstruation ($p<0.001$) and immunohistochemical staining was strongest in menstrual and proliferative endometrium. The ADM receptor was immunolocalised to vascular and lymphatic vessels. Culture of secretory, but not prolifertative, phase endometrial explants with $PGF_{2\alpha}$ or in hypoxic conditions increased ADM mRNA expression ($p<0.05$). Proliferative phase explants subjected to in *vitro* progesterone withdrawal showed increased ADM expression, but only when cultured in hypoxic conditions ($p<0.05$). Similarly, epithelial cells treated with $PGF_{2\alpha}$ or hypoxia increased ADM mRNA and protein ($p<0.05$). No changes were observed with PGE_2. Conditioned media from $PGF_{2\alpha}$ treated cells increased vascular and lymphatic endothelial cell proliferation and branching ($p<0.05$). This was abolished by co-treatment with adrenomedullin receptor antagonists. Short hairpin sequences against hypoxia inducible factor-1α (HIF-1α) inhibited ADM up-regulation by hypoxia but did not significantly alter $PGF_{2\alpha}$-induced expression. ADM and its receptor are present at the time of endometrial repair. We demonstrate that ADM is up-regulated by two distinct mechanisms involving either HIF-1 or $PGF_{2\alpha}$ pathways. Delineation of these physiological mechanisms may provide novel therapeutic targets for pathologies such as heavy menstrual bleeding.

O28 Fetal programming: direct and indirect fetal androgenisation differentially regulate the metabolic phenotype in adult sheep

K Hogg[1], AS McNeilly[2] & WC Duncan[1]
[1]Reproductive and Developmental Sciences, The University of Edinburgh, Edinburgh, UK; [2]Human Reproductive Sciences Unit, MRC, Edinburgh, UK

In *utero* androgen exposure in monkeys and sheep results in a phenotype that includes metabolic irregularities that are common in women with polycystic ovary syndrome (PCOS). However some of the fetal effects of maternal testosterone proprionate (TP) administration may be mediated by estrogen following placental aromatisation. We hypothesised that it is estrogen, rather than androgen, exposure that promotes the metabolic changes in adulthood. Pregnant Scottish Greyface ewes were injected with TP (i.m; 100mg in oil) or vehicle control (C) twice weekly from d62-102 gestation (female offspring; n=9 and 5 respectively). In a second cohort the fetus was directly injected with TP (1, 4 or 20mg) or C at d62 and d82 gestation under ultrasound guidance (female offspring; n=5, 5, 7 and 4 respectively). The effect of direct or indirect fetal TP administration on adult fat distribution and function were studied by assessing: 1) omental fat: body weight ratio, 2) histochemical assessment of fatty liver, and 3) qRT-PCR analysis of visceral fat *PEPCK* and *leptin* expression. Omental weight was increased, independently of body weight, after direct fetal TP administration ($P<0.05$) but not following indirect TP exposure by maternal administration suggesting an androgenic aetiology. Conversely, the presence of fatty liver was increased in animals indirectly androgenised by maternal TP ($P<0.05$) while direct fetal TP injection had no such effect, suggesting an estrogenic effect. However the direct fetal exposure group revealed fatty liver as a direct consequence of the injection suggesting a possible stress-induced response independent of sex-steroids. There were no differences in visceral fat *PEPCK* or *leptin* mRNA expression. These findings highlight the complexity of fetal programming of a metabolic phenotype, revealing specific effects attributed to either androgens or estrogens as well as inducers of stress responses.

Monday 12th July 2010

Distinguished Scientist
Main Auditorium
1700 - 1800

Distinguished Scientist abstract

Notes

S9 Polycystic ovary syndrome, reproduction and fertility
S Franks
Institute of Reproductive and Developmental Biology, Imperial College London, Hammersmith Hospital, London, UK

Polycystic ovary syndrome (PCOS) is not only associated with reproductive disorder (it is the commonest cause of anovulatory infertility) but also carries a greater risk of long term health problems, notably type 2 diabetes. The increasing prevalence of obesity in the population has an additional negative impact on both reproductive and metabolic sequelae of PCOS. The aetiology of PCOS remains unclear but there is compelling evidence for a major genetic disposition to the syndrome. This may appear paradoxical given that one would have expected an inherited cause of infertility to result in a dwindling population of women with PCOS. The answer to this conundrum may lie in the study of PCOS in the general population in which a wider spectrum of presentation of women with symptoms of PCOS can be observed compared with the selected population that typically presents in endocrine or fertility clinics. Such studies show that although there may be a delay in conceiving, family size is not significantly compromised overall. Indeed, it has been hypothesized that there may be a reproductive advantage in having polycystic ovaries and the attendant predisposition to insulin resistance; PCOS may represent a thrifty reproductive phenotype, thus ensuring its survival in the global population.

Koivunen R, Pouta A, Franks S *et al*, Fecundability and spontaneous abortions in women with self-reported oligo-amenorrhea and/or hirsutism: Northern Finland Birth Cohort 1966 Study. *Hum Reprod*. 2008 (9):2134-9.
Corbett SJ, McMichael AJ, Prentice AM. Type 2 diabetes, cardiovascular disease, and the evolutionary paradox of the polycystic ovary syndrome: a fertility first hypothesis. *Am J Hum Biol*. 2009 (5):587-98.

Tuesday 13th July 2010

SSR / SRF New Investigator
Main Auditorium
0900 - 1030

SSR / SRF New Investigator abstracts

Notes

S10 Regulation of Spermatogenesis: from Genetics to Epigenetics*
W Yan
Department of Physiology and Cell Biology, University of Nevada School of Medicine, Reno, USA

Male gamete production relies on successful spermatogenesis, a process through which male germ line stem cells undergo a series of differentiation programs and become sequentially spermatogonia, spermatocytes, spermatids and eventually spermatozoa. This process is clearly controlled by genetic factors, as evidenced by the fact that the human or mouse genome contains numerous testis-specific genes, many of which have been identified to be essential for sperm production. Also, mutations in some of these genes have been implicated in humane male infertility. Over the past ten years, we have identified ~500 mRNA-coding genes that are exclusively expressed in the male germ cells and display a high degree of evolutionary conservation between mice and humans. Large-scale expression profiling assays revealed that the majority of these genes are expressed in spermatocytes (meiotic male germ cells) and spermatids (haploid male germ cells). Our gene knockout studies have identified several that have an essential role in normal sperm production. I will discuss the male infertility phenotype of *Catsper3/4* and *Spem1* knockout mice, and some interesting findings relevant to clinical male infertility diagnosis and treatment.
Despite the fact that more and more spermatogenesis-essential genes are being identified, very few of them have been associated with male infertility. On the other hand, a world-wide trend of declining sperm quality over the past several decades suggests that environmental factors may also affect spermatogenesis. Such a rapid pace may imply that epigenetic factors are involved in the regulation of spermatogenesis. Recent identification of so many non-coding small RNA species (miRNAs, piRNAs, Endo-siRNAs, snoRNAs, etc.) expressed abundantly in the testis opens up a new avenue towards understanding the complex epigenetic control of spermatogenesis. I will give you an update on our progress in understanding functions of testicular small non-coding RNAs in testicular development and spermatogenesis.

*Research in the Yan lab is supported by grants from the NIH (HD060858, HD048855, HD050281, and RR18751) and the University of Nevada, Reno.

S11 The regulation of ovarian function and ovulation rate by oocyte-glycoproteins
SA Williams
Department of Physiology, Anatomy and Genetics, University of Oxford, Oxford, UK

Introduction: Modification of oocyte-glycoproteins by oocyte-specific ablation of complex *N*- and *O*-glycans has revealed new roles for the oocyte in the regulation of fertility and ovarian function.
Methods: Floxed alleles of *T-syn* and/or *Mgat1* were deleted specifically in the oocyte using a ZP3*Cre* recombinase transgene thus preventing the generation of complex *O*- and *N*-glycans respectively.
Results and Discussion: In mice generating oocytes lacking *T-syn* and thus complex *O*-glycans, ovulation rate was sustainably increased revealing an unexpected role for oocyte *O*-glycans in regulating fertility. Analysis of *T-syn* mutant ovaries revealed increased numbers of follicles although follicle atresia was not decreased. Interestingly, prepubertal mutant females treated with exogenous gonadotrophins had a reduced ovulation rate. Therefore, we have proposed a model of slowed follicle development resulting in follicle accumulation in *T-syn* mutants. Deletion of *T-syn* also resulted in the generation of numerous multiple-oocyte follicles (MOF). However, MOFs do not ovulate multiple eggs and thus do not contribute to the increase in fertility. These MOFs are generated by follicles joining predominantly at the preantral stage revealing a role for oocyte-glycoproteins in the regulation of follicle integrity. Mice generating oocytes lacking both *T-syn* and *Mgat1* (double mutants; DM) undergo premature ovarian failure by 3 months of age. DM female fertility was markedly reduced with only 30% producing a single small litter. By 12-week, gonadotrophin levels were elevated, ovarian steroids were decreased and ovaries were almost completely devoid of growing follicles. These mice have revealed new roles for oocyte-generated glycoproteins in the regulation of ovulation rate, follicle integrity and ovarian function. (This research was supported by both the NIH and the MRC).

Tuesday 13th July 2010

Symposium III: Animal models: how relevant are they?
Main Auditorium
1100 - 1300

Speaker abstracts

Notes

S12 Ovarian development in the zebrafish
C Peng
Department of Biology, York University, Toronto, Canada

Zebrafish, a small tropical fish, is a popular model for studying embryogenesis. It has also emerged as an excellent model system to study the control of ovarian development. In zebrafish, ovarian development is initiated at 10 days after hatching and fish become sexually mature at 3 months. Adult zebrafish have asynchronous ovaries, which contain follicles of all stages of development. Recent studies in zebrafish have provided some clues into how ovarian differentiation and the processes of oocyte growth and maturation are regulated.

Similar to mammals, zebrafish follicle development and maturation are controlled by gonadotropins and autocrine/paracrine regulatory factors. Our lab has been examining the role of activin, TGF-beta and bone morphogenetic protein-15 (BMP-15) in regulating oocyte maturation. We found that Activin is expressed in follicles and its expression is induced by LH. Treatment with activin increases oocyte maturation while activin binding protein, follistatin, blocks LH-induced oocyte maturation. These findings suggest that LH-induced oocyte maturation is mediated, at least in part, by activin. On the other hand, TGF-beta, also expressed in follicles, inhibits LH-, as well as 17alpha, 20beta, dihydroxyprogesterone (DHP)-induced oocyte maturation. Similarly, BMP-15, an oocyte secreted factor that plays critical roles in the regulation of female fertility in mammals, also inhibits oocyte maturation in the zebrafish. We demonstrated that overexpression of BMP-15 inhibits LH- and DHP- induced oocyte maturation while knockdown of BMP-15 results in an increase in oocyte maturation. Injection of BMP-15 antiserum to zebrafish results in a decrease in the number of small growing follicles that are normally insensitive to hormones and a corresponding increase in the number of mature follicles. These findings support a role for BMP-15 to inhibit precocious oocyte maturation.

S13 Developmental origins of obesity and hypertension: a role for leptin?
PD Taylor
Division of Reproduction and Endocrinology, King's College London, London UK

Leptin plays an important role in the central control of appetite, and is also involved in activation of efferent sympathetic pathways to both thermogenic and non-thermogenic tissues, such as the kidney, and is therefore implicated in obesity-related hypertension. Leptin is also thought to have a neurotrophic role in the development of the hypothalamus and we have recently reported that altered neonatal leptin profiles secondary to maternal obesity permanently alter hypothalamic structure and function. Hyperphagia and increased adiposity in the adult offspring of obese rodents is associated with region specific hypothalamic leptin resistance in the appetite regulatory pathways of the arcuate nucleus. Most recently, we have reported compelling evidence from our rodent model that maternal obesity confers persistent sympathoexcitatory hyper-responsiveness and hypertension acquired in the early stages of development. At just 30 days of age, offspring of diet-induced obese rats (OffOb) are hypertensive. These juvenile animals also demonstrated enhanced cardiovascular stress reactivity, and an increase in low/high frequency ratio of heart rate variability (HRV), indicative of increased sympathetic tone. Disturbed sympathetic control of blood pressure has not previously been reported as a consequence of maternal obesity. The baroreflex response was also abnormal in OffOb rats (90days) and may also represent a primary programmed defect secondary to maternal obesity. Our data adds to increasing evidence for developmental plasticity in the central efferent pathways of the autonomic nervous system, and further implicates the central role of the hypothalamus in 'hardwiring' a hyperphagic and hypertensive offspring phenotype in this model of maternal obesity.

Notes

S14 Animal models of human placentation
AM Carter
Cardiovascular and Renal Research, Institute of Molecular Medicine, University of Southern Denmark, Odense, Denmark

To study placental gas exchange a model that gives access to fetal as well as maternal circulation is desirable and structural differences from human placenta can be tolerated. The sheep remains an excellent model for this type of study. However, the most pressing questions in obstetrics concern preeclampsia. This disease is associated with shallow trophoblast invasion and insufficient widening and transformation of the uterine spiral arteries. Potentially these processes could be studied in the chimpanzee where we have shown that trophoblast invasion is similar to that in human placenta. Unfortunately there is a moratorium on chimp experiments. Other non-human primates differ in lack of trophoblast invasion by one of the two major routes seen in human and great apes. Nonetheless some progress has been made in developing a model of preeclampsia in the baboon. Rodents are perplexing because in some trophoblast invades much deeper than in the human (e.g. guinea pig). A rat model of preeclampsia showed deeper endovascular trophoblast invasion than in controls, the reverse of what occurs in the human disease. Finally in the mouse trophoblast invasion is too shallow to make this a serious model for preeclampsia. Instead the mouse has given important insights into maternal factors affecting uterine artery transformation. A second area of research concerns regulation of fetal growth; poor growth is linked to adult-onset cardiovascular diseases and metabolic syndrome. It should be borne in mind that many mammals deliver large litters of altricial young and events that occur in *utero* in humans are postnatalin these species (e.g. rats and mice. Guinea-pigs and other hystricognath rodents have longer gestations as of course do sheep and monkeys. There is enormous diversity in placental structure and we should continue to build on the comparative studies of E. C. Amoroso, a founding member of the Society.

S15 Prenatally androgenized female rhesus monkeys: a model for polycystic ovary syndrome
DH Abbott[1], LE Nicol[2], & DA Dumesic[1]
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Introduction: Discrete fetal androgen excess during early gestation in female rhesus monkeys promotes polycystic ovary syndrome (PCOS)-like reproductive and metabolic traits in adulthood, and endocrine and ovarian antecedents in infancy. Developmental changes promoting PCOS-like metabolic traits, however, remain unclear.
Methods: The present studies included 9 gravid female rhesus monkeys (*Macaca mulatta*) receiving 15mg testosterone propionate (TP) subcutaneously daily from 40-80 days of gestation (term: 165 days), while 5 gravid females received control (C) oil injections over the same gestational interval. Female fetuses were identified by absence of Y-chromosome in maternal blood by ~30 days of gestation. Dams, fetuses and infants up to 2 months of age were assessed. Adult females that received similar in *utero* exposures were also examined.
Results and Discussion: Mild-to-moderate glucose intolerance occurred in TP-injected dams at mid-gestation, accompanying ~5% increase in fetal head diameter and modestly increased weight gain in infant PA female offspring. Excessive insulin sensitivity, and insulin secretion relative to insulin sensitivity, were found in 1.5-month old PA infants during an intravenous glucose tolerance test. Immunohistochemical analyses of pancreata harvested from 2-month old infants indicated a greater proportion of small and diminished proportion of very large islets in PA compared to control females. The metabolic phenotype of infant PA monkeys, however, converted to adult PA traits of abdominal adiposity, diminished insulin sensitivity and secretion, increased incidence of type 2 diabetes and reduced numbers of islets per pancreatic area. These studies suggest that differential programming of insulin action and secretion may precede adult metabolic dysfunction in this nonhuman primate model for PCOS.

Tuesday 13th July 2010

Symposium IV: SRF 60th anniversary celebration
Main Auditorium
1400 - 1730

Speaker abstracts

Notes

S16 SRF: an historical perspective
D Whittingham

Abstract unavailable

S17 What future for SRF: curiosity or translational driven research in reproductive biology
DT Baird
Emeritus Professor, Centre for Reproductive Biology, University of Edinburgh, UK

The relative importance of basic research versus applied has been ongoing an ongoing debate since the formation of the SSF over 60 years ago. In recent years the insistence of Government on “value for money” has led to increasing pressure on scientists and clinicians to conduct research which has immediate applicability. This emphasis on “translational research” ignores the serendipidous nature of many discoveries which have led truly useful application eg pituitary down regulation with GnRH analogues. At the same time basic scientists feel pressurized to make overly optimistic claims about the potential benefits of there most recent research. Many of the applications of research could not ave been anticipated in advance eg at the time of a Grant Application.

This tension between basic and applied research has led to the formation of specialist societies and Journals e.g. British Fertility Society, Society for Developmental Biology where researchers feel that they will get a more critical reception. Some have even claimed that the days of societies like the SRF where work from many disciplines are presented and discussed, are numbered. But to question the future of a generalist society like SRF is to ignore the fact that probably the most difficult part of planning any research is to ask the right questions. What are the current problems? What potential applications might this finding have in animal husbandry as well as in veterinary and human medicine? Are there techniques and treatments in discipline that can be easily transferred to another? It is no accident that many of the spectacular advances in assisted reproductive medicine have come directly from Agricultural Science.

Reproduction is unlikely to become an irrelevant part of our society. Even if advances in Assisted Reproduction have made sexual intercourse an unnecessary component for reproduction, sex is likely to remain as one of the major factors driving behaviour. At a world level attempts to modify global warming are doomed to failure without simultaneously modifying regulation of the rate of increase of population. It is people and their activities which are warming the world! The SRF plays an important role in providing the evidence on which discussion of this issue should be discussed.

Notes

S18 A reproductive biologist’s view of hormones down the years
AS McNeilly
MRC Human Reproductive Sciences Unit, Queen’s Medical Research Institute, Edinburgh, UK

When the society was established there were only limited bioassays for any reproductive hormones and none that were sensitive to give accurate measurements in blood. Thus the whole intricate control of the reproductive cycles in males and females were a mystery. GLC was being established to measure steroids in urine and while pure protein hormones were available it was not until immunological methods were applied that radioimmunoassays (RIAs) emerged in the 1970s. Results from RIAs which were sensitive and specific have stood the test of time, although some important studies, e.g. the vital importance of LH pulse frequency in regulating fertility appear less well recognized currently. With proteins which required a dimer partner like FSHß and inhibin for biological activity some of the RIAs were not sensitive or specific enough and misled scientists until development of ELISAs. The accurate measurement of biologically active forms of hormones remains a challenge but this is a vital part of research. While it is very exciting to see changes in transcription of genes encoding these proteins in real time in vivo, if this does not result in modulation of circulating hormone levels then this is meaningless in terms of regulation of fertility. Cross species studies are also a challenge since commercial assays are geared to human or mouse hormones and may not cross react with the animal species in question. Comparative studies are important since the regulation of some reproductive systems are different between species and the underlying mechanisms were only unraveled with the development of suitable assays to document changes in plasma concentrations in these different species. Our knowledge of the endocrinology of reproductive processes in many species is extremely limited and will remain so if there is no interest in developing suitable assays that really measure what it says on the label.

S19 The Journey of ART until now
PR Brinsden
Bourn Hall Clinic, Cambridge, UK

The history of human IVF is a long and interesting story. It began with many theories and continued with research on in *vivo* and in *vitro* fertilisation in a number of different animal species. Finally in the 1960s the research progressed to human IVF, which culminated in the birth of Louise Brown on 25 July 1978 in England - the World’s first “Test-Tube Baby”.

Aristotle (384-322 BC) proposed the theory that children are a product of “the mingling of male and female seed”. William Harvey (1578-1657) wrote: “Ex ovo omnia”. Carl von Beer’s work on the ovaries and oocytes of bitches in 1825 was probably the most important research up until that time, which is why he is often referred to as “the father of modern embryology”. Some 40 years later, Walter Heape is credited with creating the first mammalian embryos (in rabbits). 60 years later, Chang achieved births of live rabbits following IVF.

Patrick Steptoe and Robert Edwards collaborated for ten years of research, which culminated in the delivery of Louise Brown in July 1978. The basic technique of IVF soon led to the development of new ideas. These included the cryopreservation of embryos, and later of oocytes; the development of ultrasound guided techniques to collect oocytes, and Intrauterine insemination (IUI). The most important later development was of intra-cytoplasmic sperm injection (ICSI). Oocyte donation programmes were developed from about the mid-1980s, and, in a few countries, gestational surrogacy was allowed. In 1989, Handyside performed pre-implantation genetic diagnosis (PGD) and sexed embryos. The fascinating story of human IVF spans a period of only about 40 years, but the research that led up to final success was preceded by several centuries of work on many different animal species.

Notes

S20 Transgenics: invaluable to reproductive biology
H Charlton
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Teasing out the variety of mechanisms involved in the complex inter-relationships between the brain, pituitary and gonads has, historically, depended upon the stimulation or ablation of brain centres; the surgical removal of the pituitary and gonads; the isolation of hormones; the identification of their receptors, and the production of agonistic and antagonistic drugs manipulating hormone release and action. The development of a variety of transgenic techniques has allowed us to manipulate hormone actions globally by the addition of genes. The use of suicide technology has allowed us to ablate specific cell types within a tissue and cell lines have been produced for in vitro studies by targeting expression of oncogenes. Knockout studies have advanced our knowledge and the latest methods of cell specific gene ablation has refined this area further. Serendipidous results from other fields of research have provided new insights into endocrine and other control mechanisms relevant to the general field of reproduction. There have been successful attempts to replace hormone or receptor genes in the field of gene therapy. For someone who worked in a department with a strong record in tracing neural circuits the use of transgenic manipulation to identify pathways in the brain involved in neuroendocrine control is particularly exciting. This general talk will attempt to identify one or two areas where transgenic technology has added to and amplified our knowledge of reproduction.

S21 Sperm Biology in the 21st Century
HDM Moore
Centre for Stem Cell Biology, Department of Biomedical Science, University of Sheffield, Sheffield, UK

The second half of the 20th century saw great technological advances in mammalian sperm biology resulting in the rapid development of artificial insemination techniques, cryopreservation, in *vitro* fertilisation (IVF) and micromanipulation such as intracytoplasmic sperm injection (ICSI). This increasing control seemed to make the underlying biology of sperm almost irrelevant. Nevertheless, while techniques have become ever more sophisticated, the fundamental questions remain about why and how the germ cell/spermatozoon has evolved in the way it has. Answer these questions and we will have a much better understanding of the causes of male infertility and testicular cancer and how to fundamentally address these problems. Moreover, in a world with a plague of human beings the need for new methods of male contraception is more important than ever. Specific gene and stem cell methods coupled with single cell and high throughput techniques give us the tools to tackle this biology and provide new therapies and applications. In my presentation I will give a few examples of where we have come from and where sperm biology may be heading in the future.

POSTERS

Notes

P1 The endocannabinoid signalling system in the human fetal gonad
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Introduction: The endocannabinoid system comprises neuromodulatory lipids and their receptors which are involved in numerous physiological and reproductive processes. It consists of the cannabinoid receptors CB1, CB2 (*CNR1, CNR2*) and GPR55 and the enzymes producing and degrading lipids that bind these receptors, such as fatty acid amide hydrolase (FAAH), monoacylglycerol lipase (MGLL) and diacylglycerol-lipase (DAGLα & β). We aimed to determine the levels of expression of these seven members of the endocannabinoid system within the developing human fetal gonad.
Methods: Gonads and plasma were collected from 44 fetuses obtained from electively terminated, normally progressing, pregnancies (11-20 weeks, REC 04/S0802/21), equally divided between male and female fetuses and also according to maternal cigarette use during gestation. Fetal plasma cotinine concentrations were determined by ELISA to confirm cigarette exposure. Gonad mRNA was extracted and real-time qPCR carried out to determine expression levels of *FAAH, DAGLA, DAGLB, MGLL, CNR1, CNR2* and *GPR55.* Data was analyzed with respect to gender, gestational age, and the smoking status of the mother (ANOVA or Wilcoxon Test, as appropriate).
Results and Discussion: Fetal ovarian expression of *DAGLA, DAGLB, MGLL* and *FAAH* increased significantly ($p<0.05$) across the second trimester while in the testis only *DAGLA* and *DAGLB* increased significantly. Transcript expression for *GPR55* was not detected. In the ovary *CNR1* was only detected in 4 and *CNR2* in 13 out of 22 fetuses, and neither showed any trend in expression across the second trimester. No significant effect of maternal cigarette smoking was observed, although 3 of the 4 ovaries with detectable *CNR1* were from cigarette-exposed fetuses. For *DAGLB* and *CNR1*, ovarian expression tended to be reduced by smoke-exposure. We conclude that the endocannabinoid signalling system is present in the second trimester human fetal gonad and is not disturbed significantly by maternal cigarette smoking.

P2 BMP-4 expression marks the extraembryonic mesoderm in the pig
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Introduction: Bone morphogenetic protein 4 (BMP-4) has multiple roles during development. During the early stages of embryonic development BMP-4 is detected in the extraembryonic ectoderm (ExE) of the mouse embryo, a structure that contributes to the development of the placenta. The lack of a recognisable ExE in ungulate embryos raises the question whether I) BMP-4 participates in similar developmental processes and II) if so, which cells are capable of producing it. The aim of this study was to detect BMP-4 and establish the pattern of expression in pre-gastrulation pig embryos.
Methods: Pig embryos derived from sows 11 and 13 days post insemination were collected by uterus flushing in warm PBS. Embryos at pre-streak I/II and primitive streak stages were processed for whole-mount in situ hybridization (ISH), fixed in 4% paraformaldehyde and hybridized with digoxigenin-labelled RNA probe.
Results and Discussion: The results show that BMP-4 was undetectable in pre-streak I embryos, but was detected in the extraembryonic mesoderm (ExM) cells between the epiblast and hypoblast in pre-streak II embryos. Later, in early primitive streak embryos, BMP-4 was detected in the growing ExM underlying the trophectoderm layer. Finally, in late primitive streak embryos, the ExM underlying the trophectoderm surrounding the embryo exhibited strong BMP-4 expression. The pattern of BMP-4 expression shown here demonstrates that the ExM is the main source of this cytokine during this critical stage of development. The close contact of the ExM in a restricted area of the trophoblast suggests that BMP-4 may be signalling these cells during the intense proliferation of trophectoderm during conceptus elongation. BMP-4 produced by the ExM, derived from the embryo, might act as the signal between embryonic and extraembryonic domains required for the co-ordinated growth of the conceptus. (Project and Griselda Valdez-Magana funded by Conacyt-Mexico and University of Nottingham.)

Notes

P3 Gene expression profiling of POU5F1 and CDX2 in cat oocytes and in vitro-produced preimplantation embryos

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Introduction: In mouse embryos, the interplay among two transcription factors (TF), Pou5f1 and Cdx2, plays a crucial role during establishment of a pluripotent ICM population, enveloped by differentiated TE cells. Pou5f1 and Cdx2 reciprocally inhibit each others' expression, resulting in a mutually exclusive pattern. Variation of these TFs' expression patterns has been reported among species and raises questions regarding the role of POU5F1 in maintaining /signaling pluripotency in different species. We aimed to characterize mRNA expression of POU5F1 and CDX2 in cat oocytes and in vitro preimplantation embryos.
Methods: Quantitative RT-PCR (RT-qPCR) was used to examine the level of transcripts in single oocytes and embryos at different stages (n=10/stage): germinal vesicle (GV), metaphase II (MII), 5 to 8-cell, compact morula (CM), blastocyst (B) and hatching blastocyst (HB). Oocytes were in vitro matured, fertilized, cultured and collected for RT-qPCR. To correct for any technical variation, results were normalized to the geometric mean of 3 validated references genes (YWHAZ, SDHA and GUSB). Results and discussion: Transcripts of POU5F1 were detected at all stages, consonant with findings in other species. POU5F1 transcript levels increased ~10-fold at the compact morula stage when compared to earlier stages ($P<0.001$) and remained upregulated. CDX2 transcripts were absent at the oocyte stages, and became detectable from the embryonic genome activation (EGA: 5 to 8-cell) onwards. CDX2 transcripts were most abundant in hatching blastocyst stages, exhibiting a ~4 fold upregulation when compared to earlier cleavage stages. Both results indicate a prominent role for POU5F1 and CDX2 subsequent to EGA. After initiation of blastocoel formation, the increase of CDX2 expression might suggest a concomitant downregulation of POU5F1, limiting its expression to ICM as demonstrated in other species. To support this hypothesis, we are currently examining these expression patterns in ICM and TE segments. Supported by 'Research Foundation-Flanders', aspirant 1.1.477.07N00.

P4 Expression of DNA methyltransferases 3 family (DNMT3s) in monoparental and biparental sheep embryos

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DNA methyltransferases 3 family of enzymes (DNMT3s) are essential for the establishment of DNA methylation during mammalian development. In particular, Dnmt3s are involved in establishment of methylation imprints in embryos. As the consequence, imprinted genes are expressed exclusively from one parental allele. We wanted to investigate temporal dynamics that underlie establishment of methylation in sheep embryos. To do this, we compared first, genome-wide methylation of monoparental (parthenogenotes and androgenotes) and biparental (in vitro fertilized) blastocysts and, second, the pattern of the expression of Dnmts (DNMT3a, DNMT3b and DNMT3L) in mono- and biparental zygotes and blastocysts. In vitro matured oocytes have been used to produce androgenetic, parthenogetic and fertilized embryos in vitro. Embryos have been analysed by indirect immunofluorescence using antibodies against 5-methylcytosine and against DNMT3a, DNMT3b and DNMT3L proteins. The intensity of fluorescence has analysed by ImageJ. A higher methylation signal has been observed in parthenogenetic blastocysts in comparison to biparental ones ($P<0.05$). Notwithstanding, non of DNMTs has been found to be deregulated in blastocysts of any group. Instead, both, parthenogenetic and androgenetic zygotes showed a lower expression of DNMT3a and DNMT3b compared to biparental ones ($P<0.05$), while DNMT3L was similarly expressed in all groups. In all groups of zygotes, all DNMTs proteins were detected in both nucleus and cytoplasm. In blastocysts, DNMT3a and DNMT3L proteins showed mostly a nuclear localization while DNMT3b protein was detected mainly in cytoplasm in all groups. These results showed the reduced expression of DNMT3a and DNMT3b proteins in monoparental zygotes but not in blastocysts. Furthermore, an increase of global DNA methylation was observed in parthenogenetic blastocysts. The main message that our work conveys is that the effects of unbalanced parental contribution on the embryonic epigenotype appear very early in development and lead to remarkable difference in the methylation profile already at the blastocyst stage.

Notes

P5 Expression and subcellular organization of de novo DNA methyltransferases (Dnmts) in adult and prepubertal oocytes and embryos in sheep

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Mammalian Dnmts: Dnmt3a, Dnmt3b together with the accessory protein Dnmt3L establish DNA methylation patterns in the genome during gametogenesis and embryogenesis. The regulation of Dnmts expression during oocyte/embryo development has been studied mainly in mouse. However, because of species specific differences in DNA methylation/demethylation during embryogenesis it is necessary to know how the expression of these enzymes is regulated in other mammals. We aimed to study the pattern of Dnmts expression in oocytes and embryos of large animal, the sheep. As a negative model we also analysed the Dnmts expression in prepubertal sheep oocytes and embryos, in which we previously revealed DNA hypomethylation. We compared the expression of Dnmts in in vitro cultured adult and prepubertal sheep oocytes (germinal vesicle, GV and metaphase II, MII) and preimplantation embryos (zygote and blastocyst) by immunocytochemistry using polyclonal antibodies. Samples were observed under fluorescent microscope and signal intensity was analysed. Dnmt3a was mainly localized in cytoplasm in GV, MII and zygotes while a strong nuclear signal has been observed in blastocysts. The strongest Dnmt3a signal has been observed in GV, than remained constantly lower till the blastocyst. Additionally, a deregulated expression ($p<0.01$) of Dnmt3a in prepubertal oocytes (GV, MII) but not in embryos has been noted. Expression of Dnmt3b was detected constantly at similar intensity, in cytoplasm either in adult and prepubertal models. The expression of Dnmt3L was cytoplasmic and the relative expression was higher ($p<0.001$) in oocytes then in embryos. Interestingly, the relative expression of Dnmt3L was significantly higher ($p<0.04$) in prepubertal MII oocytes while significantly lower ($p<0.04$) in prepubertal blastocysts in comparison to adult counterparts. Together, these results provide a better understanding of the developmental regulation of Dnmts during oocyte and embryo development in sheep. The use of prepubertally derived oocytes and embryos emphasises age dependent establishment of Dnmts expression pattern.

P6 Protein and mRNA variants of centromeric protein F (CENPF) in bovine preimplantation embryo

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Introduction: CENPF plays crucial role in microtubule-kinetochore interaction and chromosome condensation. We found that the majority of embryos with silenced CENPF mRNA arrest after EGA. Using immunofluorescence we found that protein silencing is detectable only when using antibody targeted to the C-terminus of the protein. This suggests existence of more CENPF variants. The aim of the study was to identify CENPF splicing variants expressed in preimplantation bovine embryos.
Methods: The alternative exons were identified by computational and experimental approach. The candidate alternative exons were predicted based on interspecies conservation or we verified the Ab-initio predicted alternative exons from Ensembl. The alternatively spliced exons were verified in both bovine embryonic and fibroblastic mRNA using RT-PCR, gel electrophoresis and sequencing. Bovine oocytes were obtained from abattoir-derived ovaries, in vitro matured, fertilized and cultured. The protein variants were verified by western blot using two different antibodies against N- and C- termini of CENPF.
Results and Discussion: The full-length mRNA was present in both embryos and fibroblasts. However, the full-length protein was detected almost only using the N-terminal antibody in embryos. This result suggests a modification of the C-terminus, which is necessary for degradation of the protein. We found that CENPF is not degraded in pre-EGA embryos and we therefore assume that the lack of degradation may be related to the C-terminal changes. We established new alternative exons in both kinetochore-binding regions of CENPF – between exons 13 and 14; 17 and 18. Kinetochore binding is one of the most important roles of CENPF. Currently it seems that the previously noticed differences in CENPF expression and function in individual species were result of distinct methodical approaches. The acquired results may hence bestead not only in preimplantation embryology but also in medicine where CENPF is used as a proliferation marker. Supported by GACR 523/09/1035 and 204/09/H084.

Notes

P7 Intrinsic oxidative stress in SOD1-deficient embryo under atmospheric oxygen culture causes developmental arrest without mitochondrial malfunction

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Introduction: Among factors that have a deteriorating effect on fertilizability and subsequent developmental competence in in vitro-produced embryos, oxidative stress is a well-known cause of developmental arrest, cell death by necrosis or by apoptosis, suppression of sperm motility, and sperm-oocyte fusion. To clarify a cause of abnormal development by oxidative stress, we investigated the effects of intrinsic oxidative stress on the early development of embryos that were resulted from in vitro fertilization (IVF) or in vitro maturation-fertilization (IVMF) of superoxide dismutase 1 (*SOD1*)-deficient mouse oocytes.
Methods: Superovulated or in vitro matured cumulus-oocyte complexes (COCs) derived from *SOD1*-deficient and wild-type mice were fertilized with spermatozoa from wild-type mice and were cultured for 4 days. We measured the levels of intracellular superoxide by dihydroethidium staining, the ATP content, and mitochondrial membrane potential using JC-1 in in vitro-matured MII oocytes and IVF embryos.
Results and Discussions: Development of all IVF embryos from *SOD1*-deficient oocytes was arrested at the two-cell stage under atmospheric oxygen culture (20% O_2). Also, most of the IVMF embryos from *SOD1*-deficient oocytes were not cleaved to two-cell stage nevertheless high frequency in sperm penetration under 20% O_2. Significantly higher levels of superoxide were detected in these arrested embryos. While treatment with antioxidants was unsuccessful, hypoxic culture (1-5% O_2) of IVF *SOD1*-deficient embryos rescued the two-cell arrest and ameliorated impairment in fertilization of IVMF *SOD1*-deficient embryos. Mitochondrial function was investigated because its malfunction was a suspected cause of embryo arrest. However, ATP content and mitochondrial membrane potential in *SOD1*-deficient embryos were not distinctly decreased as compared with wild-type embryos under 20% O_2. These results suggest that the mitochondrial function of ATP production via oxidative phosphorylation was almost normal in the *SOD1*-deficient embryos and that the mechanism of cell cycle regulation might be a target of elevated ROS.

P8 Profiles of pluripotency and differentiation factors in In Vitro Fertilized (IVF) embryos and their reprogramming in cloned embryos: a special perspective to Embryonic Genome Activation (EGA) in Bovine

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In nuclear transplantation (cloning) oocyte 'reprograms' differentiated nucleus into an embryonic state, a phenomenon equivocal to maternal to embryonic transition (MET) in normal fertilization. Both of these genomic transitions ultimately result in EGA. However, clones have low birth rate compared to fertilized embryos. Recent advances in direct reprogramming through ectopic expression of pluripotency associated transcription factors have emphasised their role in nuclear reprogramming. Nevertheless, there is no study in bovine, which describes the expression profiles of pluripotency transcription factors during MET in normal embryos and their reprogramming in clones. In order to understand nuclear reprogramming an earlier assessment of embryos/clones is important which is possible immediately after EGA. We hypothesized that the acquisition of a proper gene expression of pluripotency and differentiation factors by the cloned morulae (first stage after EGA in bovine) could be an early indication of appropriate nuclear reprogramming. Therefore, the objectives of this study were a) to chart the expression profiles of important pluripotency and differentiation factors during preimplantation stages of bovine IVF embryos and b) early assessment of the nuclear reprogramming by comparing cloned morulae with IVF controls. In this study we profiled pluripotency factors: OCT4, SOX2, NANOG and STAT3, as well as differentiation factors: HESX1, NODAL, ISL1, ZIC3 and MEIS1 through quantitative RT PCR. We analysed six preimplantation stages including immature and mature oocytes, 4 cell, 8 cell, morula and blastocyst. Moreover, the cloned morulae derived from two donor cell lines with different term development potentials (1.8% vs. 12.7%) were analysed in comparison to donor cells and IVF morulae. This study reveals that the EGA is an important phase which involves both: the silencing of genes of differentiation and the activation of genes of pluripotency—significant for normal embryonic development. Additionally, cloned morulae from both cell lines have undergone significant reprogramming by stage morula.

Notes

P9 Distribution of cytoskeletal proteins and the basement membran component laminin in 16 day-old horse embryos

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Introduction: The aim of the present study was to determine the distribution of cytoskeletal proteins in the various cells of the different germinal layers in 16 day-old equine conceptuses (somite stage). Furthermore, the development of a basement membrane at this stage of equine embryonic development should be investigated.
Methods: Equine conceptuses (n=2) were flushed, fixed in 4% formaldehyde and embedded in paraffin. Sections of the embryonic disc, the border of the trilaminar omphalopleure and the bilaminar trophoblast were stained immunohistochemically for cytokeratin, vimentin, desmin, tubulin, and laminin.
Results and Discussion: In equine embryos on day 16, mesoderm expansion reached far beyond the embryonic disc building the trilaminar omphalopleure, the other parts of the trophoblast remaining bilaminar. A differentiation of the lateral mesoderm into a parietal (somatic) and a visceral (splanchnic) lamina was observed. Tubulin was found in all cells of the developing embryo, most intense staining occurred in the apical region of the neural groove cells. In the bilaminar trophoblast region entodermal cells showed stronger immunoreaction to anti-tubulin than the ectodermal layer. Vimentin was expressed in the mesodermal cells (somites and lateral mesoderm). In addition, the basal region of the neural groove cells was positive for vimentin, while the ectodermal and entodermal layer was not stained in any region. Cytokeratin immunostaining was observed in all cell types including mesodermal cells. Desmin was present in the mesoderm of the trilaminar trophoblast, but only weakly stained in the other mesodermal regions. A basement membrane as determined by laminin staining was observed under the cells of the neural groove, ectodermal and entodermal cell layers. The mesodermal region showed an interrupted basement membrane or expression within the mesodermal cells (trilaminar trophoblast). Further studies with several developmental stages of equine embryos are neccessary to completely determine the spatial expression of cytoskeletal and basement membrane proteins.

P10 Gonadotrophin subunits in the pituitary gland of fetal cattle

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Introduction: In sheep, the number of pituitary gonadotrophs containing LHβ and FSHβ subunits reaches a peak during mid-pregnancy followed by a decrease towards the end of pregnancy, a pattern mirrored by circulating gonadotrophin concentrations. The aim of these experiments was to describe the percentage of pituitary gonadotrophs containing and expressing LHβ and FSHβ and their peripheral concentrations in fetal cattle.
Methods: Bovine fetal pituitary glands were dissected and either fixed for immunofluorescence staining (LHβ or FSHβ; n=26) or snap-frozen for gene expression analysis (α-gonadotrophin subunit (α-SU), LHβ, FSHβ and GnRH receptor (GnRHR); n=14). Blood samples were assayed for peripheral gonadotrophin concentrations. Fetal age was estimated using crown-rump length and fetuses were grouped into 1st (<90 days (d); n=4); early 2nd (91-150d; n=6) and late 2nd (151-180d; n=3); and 3rd (>210d; n=3) trimesters of gestation. Normality was evaluated and data were transformed where necessary, prior to statistical analysis using one-way ANOVA.
Results and Conclusions: The percentages of gonadotrophs containing LHβ and FSHβ were similar ($P>0.05$) between male and female fetuses across all ages, increasing ($P<0.05$) towards parturition. Contrary to this, peripheral concentrations showed a different trend which varied depending on sex, with female fetuses showing the highest ($P<0.05$) concentrations during late 1st trimester (<90d), with lower concentrations thereafter. In males, gonadotrophin concentrations were similar throughout the 2nd ($P>0.05$) followed by an increase ($P<0.05$) during the 3rd trimester of gestation. Expression of α-SU, LHβ and FSHβ subunits and GnRHR was first observed between 65d and 75d of gestation. The above experiments showed that the hypothalamo-pituitary axis is active during early stages (<90d) of fetal development in cattle. The observed sex-differences in pituitary and peripheral gonadotrophin concentrations may correspond to the differential sexual regulation of gonadotrophin synthesis and release during fetal life in cattle.

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P11 Testosterone like activity of Mucuna pruriens and its effects on serum biochemical metabolites in immature male rats

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Introduction: Traditionally, *Mucuna pruriens* has been used as carminative, a hypoglycaemic agent, to increase libido and for the treatment of Parkinson's disease. In the present study, testosterone like activity of the ethanolic and aqueous extracts of seeds of *Mucuna pruriens* was investigated. Moreover, its effects on serum biochemical metabolites were also monitored.
Method: The extracts of dried seeds of *Mucuna pruriens* were prepared in 95% ethanol and water using Soxlet's apparatus.Thirty six immature male Sprague-Dawley rats (3-4 weeks of age) were divided into three equal groups A, B and C. Rats of groups A and B were orally given ethanolic and aqueous extract of Mucuna pruriens @ 500 mg/Kg body weight, respectively, for 14 days. Rats of group C served as untreated control. The experimental rats were euthanized after 14 days of treatments and blood samples were collected from each rat were used for serum biochemical analysis.
Results and Discussion: Rats of group A gained higher body weight compared to those of groups B and C ($P<0.05$). Serum testosterone, total proteins, total cholesterol and HDL cholesterol concentrations were higher in rats of groups A and B compared to those of group C. However, there were no differences in serum triglycerides, LDL cholesterol and ALT activity among rats of three groups. Serum urea concentration and AST activity were lower in rats of groups A and B than the control rats. It was concluded that both ethanolic and aqueous extracts of seeds of *Mucuna pruriens* possessed testosterone like activity; and short term treatment of rats with these extracts increased serum total proteins and total cholesterol, the latter was mainly due to increased HDL concentrations. Moreover, these extracts had no adverse effects on the liver or kidney functions. (Financial support for this study was provided by the Pakistan Agricultural Research Council, Islamabad).

P12 Circulating melanin concentrating hormone (MCH) concentrations in the menstrual cycle

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Food intake regulation and energy expenditure are the two most studied roles of the neuropeptide MCH to date. The source(s) and role(s) of circulating MCH are unknown. Both food intake and energy expenditure change throughout the menstrual cycle. It was the aim of this study to determine if circulating MCH concentrations change over the menstrual cycle. The menstrual cycles of 26 women (mean ± sd: 36.1 ± 6.0 years old; BMI, 24.6 ± 5.2 kg/m^2) were monitored by following their menses, awakening body temperatures and plasma progesterone concentrations. Resting metabolic rates (RMR) were measured and a fasting blood sample taken in the early follicular, late follicular, early luteal and late luteal phases. MCH, leptin and progesterone concentrations were determined in each plasma sample by radioimmunoassay. Ovulation appeared to have occurred in 20 of the women. There were no differences in circulating MCH concentrations throughout the menstrual cycle however leptin concentrations were lower in the early follicular phase compared to the early and late luteal phases ($p < 0.05$). RMR was lower in the late follicular phase compared to the early and late luteal phases in the ovulatory women ($p < 0.05$) whilst RMR remained the same over the cycle in non-ovulatory women. Based on rodent studies if there was a relationship between hypothalamic MCH expression and circulating MCH concentrations, it would be predicted that when circulating MCH concentrations decrease, RMR would increase. The results of this study would suggest that there is no strong association between circulating concentrations of MCH and RMR over the menstrual cycle.

P13 Discovery of novel Escherichia coli strains from cattle with metritis that are pathogenic for the bovine endometrium

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Introduction: About 40% of dairy cows develop metritis or endometritis after parturition and these diseases cause infertility. Infection of the endometrium with *Escherichia coli* after parturition is the first step in establishing disease. It was assumed that the endometrium is infected by random genetically diverse strains of *E. coli* from the environment. This study tested the alternative hypothesis that distinct strains of *E. coli* are pathogenic for the bovine endometrium.
Methods: Sixty four dairy cows were monitored for uterine health each week post partum and bacteria isolated from the uterine lumen. The genetic relationships between 114 strains of *E. coli* were determined using molecular genotyping methods, including multilocus sequence typing. The pathogenicity of *E. coli* strains was explored in *vitro* by measuring bacterial adhesion and invasion of bovine endometrial cells. The secretion of prostaglandins and interleukin-8 was measured to evaluate the response of the endometrial cells to lipopolysaccharide purified from the bacteria.
Results and Discussion: Distinct clonal groups of endometrial pathogenic *E. coli* (EnPEC) were identified by multilocus sequence typing from animals with metritis. The EnPEC genotypes differed from reference enteric or extra-intestinal pathogenic bacteria, and EnPEC did not express the expected virulence genes associated with other pathogenic *E. coli*. However, EnPEC were more adherent and invasive for endometrial epithelial and stromal cells, compared with *E. coli* isolated from clinically unaffected animals. Stromal cells secreted more prostaglandin E_2 and interleukin-8 in response to lipopolysaccharide purified from EnPEC than *E. coli* from unaffected animals. Epithelial cells not only secreted more interleukin-8 but surprisingly switched prostaglandin secretion from the expected F series to the E series, via a phospholipase A2-mediated mechanism. The implication arising from the discovery of EnPEC is that development of treatments for endometritis should focus on EnPEC and not other strains of bacteria. (Supported by BBSRC.)

P14 The effect of lipoprotein-delivered n-3 and n-6 polyunsaturated fatty acids on ovine follicular-cell steroidogenesis and in vitro embryo development

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Introduction: We previously found increased follicular-fluid progesterone concentrations in ewes fed an n-3 polyunsaturated fatty acid (PUFA)-enriched diet, but detected no differential effect of n-3 and n-6 PUFA-HDL on granulosa-cell (GC) steroidogenesis. Here we tested the hypothesis that n-3 PUFA-mediated steroidogenesis occurs in thecal cells (TC) and assessed the effects of n-3 and n-6 LDL and albumin on ovine embryo development.
Methods: HDL, LDL and albumin were derived from sera of ewes fed n-3 or n-6 PUFA-enriched diets. GC and TC from large-antral follicles were plated at $3.5x10^5$ and $2.0x10^5$ cells per well, respectively, in TCM199 (GC) and DMEM-F12 (TC). Lipoproteins were added in a 2x2 factorial arrangement (n-3 vs n-6; HDL vs LDL) at 72µg/ml fatty acid (FA) equivalent, and cells cultured for 6 days. Steroids in medium were measured by ELISA. Day 1 zygotes were cultured for 6 days in SOFaa with one of five supplements (1) FA free-BSA, n-3 (2) or n-6 (3) LDL, n-3 (4) or n-6 (5) albumin. Lipoproteins were added at 14µg/ml FA equivalent.
Results and Discussion: TC number increased ($P<0.001$) during culture and was greatest ($P=0.02$) for n-3LDL. In contrast, progesterone concentration (ng/10^5 cells) was greatest ($P=0.009$) for n-3HDL. Neither GC number nor GC steroid production was affected by treatment. LDL had no effect on either Day 6 or Day 7 blastocyst yields relative to FA free-BSA. In contrast, both n-3 and n-6 albumin increased ($P<0.001$) Day 7 blastocyst yields relative to FA free-BSA (45.0 and 40.2 vs 25.3%). Furthermore, relative to LDL, albumin increased ($P<0.001$) Day 6 (37.4 vs 14.7%) but not Day 7 (42.6 vs 36.0%) blastocyst yields. In conclusion, n-3HDL increased progesterone production relative to n-6HDL in TC but not in GC. FA-enriched-albumin advances pre-implantation embryo development relative to LDL, but there is no differential effect of n-3 and n-6 PUFA.

Notes

P15 Wean-to-service interval as an indicator of pregnancy loss during the seasonal infertility period in pigs

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Introduction: Poor oocyte developmental competence is associated with lower ovarian progesterone during seasonal infertility. Wean-to-service interval (WSI) is also a risk factor for late pregnancy loss during this time. The aim of this study was to determine if WSI was associated with seasonal changes in ovarian morphology and oocyte developmental competence in sows experiencing late pregnancy loss. **Methods:** Ovaries were collected in winter from sows that were mated in summer but found to lose their pregnancies after Day 35 post-mating. Sows were grouped according to their previous WSI and the presence or absence of corpora lutea (CL). The same was repeated for ovaries collected in late-spring from sows mated in winter. Groups were WSI<6d/CL, WSI<6d/NCL, WSI>6d/CL and WSI>6d/NCL. Cumulus-oocyte complexes were recovered from antral follicles (3–8 mm) and subjected to parthenogenetic activation following in vitro maturation during winter (n=584) and late-spring (n=612). Data were analysed using a generalised linear mixed-model in GenStat release 10. **Results and Discussion:** The proportion of oocytes developing to the blastocyst stage in winter was higher than in late-spring (64% vs 34%; P<0.001). Interestingly, oocytes from sows with longer WSI had a greater ability to form blastocysts than those with shorter WSI (62% vs 38%; P<0.001). The proportion of oocytes forming blastocysts was not affected by the presence/absence of CL. Our results suggest that WSI is not associated with seasonal changes in oocyte quality during seasonal infertility. It appears those animals culled for pregnancy loss that were mated in the seasonal infertility period and later lost their pregnancies, had superior oocyte developmental competence by the time slaughter occurred in winter. Whereas those oocytes from sows culled for pregnancy loss in late-spring are less able to reach their full developmental potential when approaching the seasonal infertility period. (This work was supported by the Australian Pork CRC).

P16 Photoperiod and nutrition interact centrally to modulate the seasonal pattern of reproduction in sheep

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Most mammals from temperate latitudes have seasonal patterns of reproductive function varying between active and quiescent gonadal states. Photoperiod is the main environmental factor responsible for this pattern but other cues such as nutrition may modify photoperiodically determined seasonal patterns of reproduction. We report here an interaction between photoperiod and nutrition on seasonal patterns of ovarian activity and LH secretion in the ewe. We also assessed if nutrition affected melatonin secretion. Thirty-one mature Île-de-France ewes were fed contrasting diets to produce two groups of ewes; well-fed (WF, median BCS=3.0; n=19) and restricted (R, median BCS=1.8; n=12). Half of the animals from each group were ovariectomized (OVX) and received oestradiol replacement treatment. Ovarian activity and LH concentrations were monitored by measuring progesterone or LH in blood plasma collected twice weekly by jugular venipuncture. Melatonin secretion was determined every hour for 24h at the summer solstice. Data are presented as means ± sem and analysed by one-way ANOVA. Entire ewes in the WF group had a longer season of ovarian activity compared to R ewes (169±8 v.s. 81±18 days; p<0.01). In parallel, the R-OVX group had a delayed onset of LH secretion compared with the WF-OVX group (14-Aug±6d v.s. 05-Sep±3d; p<0.01). There was a difference between groups in night-time amplitude of melatonin (WF: 165±32 pg/mL, R: 71±13 pg/mL; p<0.05). These results suggest that nutritional and photoperiodic factors interact at the hypothalamo-pituitary level leading to altered patterns of seasonal ovarian activity and that the site of this interaction includes a component upstream of the integration of photoperiodic signals with melatonin secretion. (RJS is the recipient of an EU Marie Curie Chair of Excellence MEXC-CT-2006-042499 and JBM a PhD grant from the “Ministère de l’Enseignement Supérieur et de la Recherche”).

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Notes

P17 Deregulated expression of DNA methyltransferases (Dnmts) and of imprinted genes in prepubertal sheep metaphase II (MII) oocytes

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Ovaries from prepubertal females represent a large reserve of gametes useful in reproduction programs. Notwithstanding of the ability of prepubertal oocytes to progress through meiosis, only a little proportion of fertilized oocytes is able to complete their development in utero. We observed genome-wide hypomethylation of GV and MII oocytes from one-two months old prepubertal sheep. In order to found specific causes of global hypomethylation of DNA in prepubertal eggs we estimated the expression of specific Dnmts required for de novo methylation of imprinted genes and for the maintenance of methylation at genomic imprints. Following mRNA extraction from MII oocytes derived from prepubertal and pubertal (control) oocytes the relative level of transcription of de novo Dnmts: Dnmt3a, Dnmt3b and Dnmt3L as well as of maintenance Dnmt1 was analyzed by quantitative Real-Time PCR. The relative level of mRNA transcription of Dnmt3a and Dnmt3L and Dnmt1 was higher (P<0.005 Mann Whitney t-test) in prepubertal eggs. The high mRNA level of de novo Dnmts corresponded with their high protein content, as confirmed by our immunocytochemical analysis. In order to further check if deregulation of Dnmts is influencing the establishment of maternal primary imprinting in prepubertal MII oocytes we analyzed also the expression of imprinted genes in MII oocytes from prepubertal and pubertal sheep. Our results show a deregulated expression (P<0.05 Mann Whitney t-test) of imprinted genes: Peg1/Mest, Igf2r and Snrpn. Obtained data suggests that notwithstanding of apparent readiness to start developmental program exhibited by the acquisition of MII stage, prepubertal sheep egg appears to be epigenetically unprepared in term of establishment of genome methylation. In particular, the failure to establish primary imprinting in prepubertal eggs can be the primary cause of their frequent developmental failure occurring throughout the first half of gestation.

P18 Aberrant methylation of oocytes and embryos derived from prepubertal sheep

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Oocytes collected from prepubertal females has reduced developmental potential. In addition to well known cytoplasmic immaturity of these gametes, we reported the nuclear failure of prepubertal sheep oocytes. In order to check the nature of nuclear immaturity we wanted to verify first, global DNA methylation in prepubertal oocytes and blastocysts and second, the level of expression of DNA methyltransferase 1 (DNMT1), which is the major enzyme responsible for maintenance of methylation during DNA replication. We matured and fertilized in vitro oocytes collected from slaughtered 2 months old lambs and cultured embryos using our routine protocols. Metaphase II oocytes and blastocysts from prepubertal and control adult sheep were evaluated for global methylation by anti-5-methylcitosine antibody. mRNA level of DNMT1 was evaluated by real-time PCR. The result show that global methylation of metaphase plates obtained from prepubertal oocytes is significantly lower (P=0.002) than adult oocytes. The low level of methylation (P=0.0012) was also maintained in blastocysts derived from prepubertal oocytes. Particularly, the methylation signal was lower in trophectodermal cells of prepubertal embryos (P=0.0068). Furthermore, we detected higher expression (P<0.05) of DNMT1 either in prepubertal oocytes and blastocysts. This interesting discrepancy between low DNA methylation and high level of DNMT1 expression in prepubertal embryos suggests to examine the expression of proteins that interact with DNMT1 and regulate the DNA methylation. In conclusion, these results support the epigenetic immaturity of prepubertal methaphase II oocytes and embryos, which may contribute to reduced developmental competence of prepubertal female gamete.

Notes

P19 Does ultrasound underestimate the number and size of ovarian follicles?

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Introduction: Assisted conception units regularly use ultrasound to estimate ovarian reserve and track follicles during controlled ovarian stimulation, in order to predict and measure ovarian response respectively. Measurements of follicle number and size are conventionally performed manually using 2D methods, but 3D ultrasound has allowed the development of automated techniques such as "sonography-based automated volume count" (SonoAVC™: GE Healthcare, Zipf, Austria), which is becoming increasingly used in clinical practice. The aim of this *in vitro* study was to examine the detection limit and accuracy of this novel method for both the small antral follicle population, in addition to the larger follicles.
Method: 24 bovine ovaries, which are morphologically similar to those of the human, were insonated through a tissue barrier using a 3D ultrasound machine (Voluson E8 Expert: GE Medical Systems). Following 3D data acquisition, SonoAVC™ was used to automatically identify and measure each and every follicle. Antral follicles measuring ≥10mm were then dissected from the ovaries, then counted and their size estimated while submerged in order to obtain as true a spherical diameter as possible. Follicles measuring ≤10mm were used for antral follicle count examination (n=668) and follicles measuring >10mm were individually identified for use in the follicle-tracking part of the study (n=32). Data were statistically analysed by Chi-squared and paired t-testing respectively with significance set at $P<0.05$.
Results and Discussion: The automated SonoAVC™ antral follicle count significantly underestimated follicle number ($P<0.001$) by 45.8% (≤2mm), 40.1% (2-3mm), 65.4% (3-4mm), 46.15% (4-5mm) and 8.7% (5-10mm). All follicles of >10mm were detected. The software also significantly underestimated large follicle diameter by an average of 18.5% ($P<0.001$). These results show that SonoAVC™ underestimates antral follicle number of follicles <5mm, and whilst accurately detecting larger follicles, the software underestimates follicular diameter in this cohort, and therefore care must be taken by the clinician in interpreting SonoAVC™ results.

P20 Suitability of vaginal impedometry and transabdominal ovarian ultrasonography for monitoring periovulatory events in the bitch

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Introduction: There is presently no technique to accurately predict ovulation and to non-invasively determine the ovulation rate in the bitch. The main objectives of the present study were hence to relate the: i) vaginal mucous impedance (VMI); ii) ultrasonographically determined follicle numbers and sizes; and iii) ovarian blood flow to the occurrence of the preovulatory LH peak. In addition, we compared daily numbers of antral follicles recorded during the periovulatory period with the number of corpora lutea (CL).
Methods: Twelve beagle and hound-cross bitches were examined daily from the onset of proestrus until ~4 days post-LH surge. Real-time B-mode and color Doppler ultrasonography utilized a 7.5-MHz transabdominal transducer connected to the ALOKA-SSD3500 echo camera. VMI was measured with a two-electrode impedometer (DRAMIŃSKI®). Serum LH concentrations were analyzed by validated radioimmunoassay. Ovariohysterectomy was performed between days 11-15 post-LH peak. The institutional Animal Care Committee gave ethical approval for this research.
Results and Discussion: Daily VMI values were indicative of the LH peak in only 45.6% of animals (95% confidence interval, 20.0% to 73.5%). There was a significant ($P<0.05$) increase in ovarian blood flow from the day of to 1 day after the LH peak. No changes ($P>0.05$) were noted in daily numbers or sizes of follicles ≥2 mm relative to the day of LH peak. There were no correlations between numbers of follicles and CLs. In conclusion, VMI was not indicative of the LH peak. Ultrasonography did not permit the estimation of ovulation rates. However, transabdominal ovarian ultrasonography did permit the precise detection of the LH peak in dogs through the visualization of increasing ovarian blood flow.

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Notes

P21 Leptin mRNA originates from canine uterus and placenta

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Introduction: In cyclic bitches endometrial epithelial cells (EECs) undergo morphological and biochemical changes during the oestrous cycle. In late metoestrus foamy EECs accumulating fat appear and during early anoestrus they are in process of desquamation. Regeneration of the epithelium is shown during mid anoestrus by simple cuboidal epithelial cells. It is hypothesized that epithelial desquamation is a protective mechanism against bacteria, which can easily enter the uterus during oestrus and cause pyometra. Foamy cells are also observed in glandular chambers of the canine placenta. The specific function of these cells is still unclear. The present study hypothesized that leptin, an adipocyte hormone important in inflammation, immune function, regulating energy homeostasis and reproduction, is associated with the appearance of foamy EECs.
Methods: Uterine tissue featuring foamy EECs was obtained from 5 healthy bitches undergoing ovariohysterectomy; together with placental and canine fat tissue, qPCR was used to verify leptin-mRNA expression. Electron microscopy was used to study cell morphology. Immunohistochemistry was adopted to investigate expression of steroid hormone receptors and proliferation activity.
Results and Discussion: Using qPCR leptin-mRNA could be detected in samples of placenta, uterine tissue featuring foamy EECs and fat but not in uterine tissue lacking foamy EECs. Estrogen and progesteron receptor expression was marginal in foamy EECs, low mitotic activity was present. Electron microscopy of foamy EECs showed no signs of apoptosis but physiological cell morphology. This study indicates that there is a link between the appearance of the foamy EECs and leptin-mRNA expression. As foamy EECs also appear in pathological situations the data reported in this study will be helpful for further studies concerning the role of leptin in physiological and pathological conditions of the canine reproductive tract. The next step is to determine the source of leptin-mRNA using laser capture microdisssection.

P22 Optimization of a three-dimensional in-vitro mouse implantation model

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The lack of an appropriate model system is one of the obstacles in implantation research. Here, we developed a three dimensional model for implantation research. The advantage of the model was that it reflected the in-vivo situation better. The endometrium was dissected from day four pregnant ICR mice. A metal mesh was used as a support for culturing the endometrium. First, we examined the use of human amniotic membrane as an additional support. Second, we compared the use of 30% fetal bovine serum (FBS) and 1.2% bovine serum albumin (BSA) in DMEM/F12 medium in culturing the endometrium. Third, we studied the effect of FBS supplemented with 63.5 nmol/L progesterone and different amounts of estrogen. The endometrium was cultured in the above conditions for 24-, 48- and 72-hours before histological examination. We compared epithelial thickness (ET), glandular to stroma area ratio (G/S) and cell density (CD) to its corresponding in-vivo controls (tissues from appropriate psuedopregant mice). Among the 3 morphological parameters, there were significant differences of ET and CD between fresh day 4 mice endometrium and endometrium cultured without amniotic membrane for 24 hours besides, degeneration of the endometrium was obvious after 48 hours of culture. Endometrium cultured in FBS medium was not different from its control after 48 hours of culture. The medium containing 30% FBS, 0.9 nmol/L estrogen and 63.5 nmol/L progesterone was best in maintaining endometrial morphology. Around 50% of the blastocysts attached to the cultured endometrium after 28 hours of co-culture in this optimized condition, and the attachment rate is significant higher than the same medium without steroid hormone. The morphological parameters in the optimized condition remained similar to the corresponding in-vivo control for 48 hours. The system provides a good in-vitro model for studying endometrium physiology and embryo implantation.

P23 Abnormalities of reproduction in captive female great apes: the impact of contraception and ape origin

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Introduction: Female captive non-human primates, including the great apes (Hominidae family), exhibit reproductive dysfunction including endometriosis, cystic ovaries, uterine polyps, abnormal bleeding, abortion and offspring mortality. For this project, it was hypothesised that these abnormalities have an environmental aetiology. This study examined the impact of contraception, age, indices of stress (rocking, hair plucking) and ape origin (wild versus captive born) on reproductive wellbeing.
Methods: Animal Record Keeping System (ARKS) records and Medical ARKS (MedARKS) records were collected from 9 zoological collections and analysed for 120 female great apes (Gorilla gorilla gorilla, n=54; Pan paniscus, n=6; Pan troglodytes, n= 43; ¬Pongo pygmaeus pygmaeus, n=17). Data were analysed by binary logistic regression and Fisher's exact test.
Results and Discussion: Reproductive abnormalities occurred in 11.7% of surveyed apes varying from 9.3% in G. gorilla gorilla to 17.6% in P. pygmaeus pygmaeus. Eleven different pathologies were observed with the most common being cystic ovaries (21.5% of affected apes). Contraception had no effect on the incidence of the reproductive abnormalities investigated. Older apes (≥25 years) were significantly more likely to exhibit an abnormality than younger animals (p=0.003) and the mean age at diagnosis was significantly lower in P. pygmaeus pygmaeus (8.7 years) than G. gorilla gorilla (25.8 years) (p=0.022). Significantly more G. gorilla gorilla (16.7%) than P. troglodytes (2.3%) exhibited stress behaviours (p=0.03). Offspring mortality was 32.7% of all births, with only 55% of P. pygmaeus pygmaeus births surviving longer than one week post partum, compared to 90% in P. paniscus. Interestingly, wild caught apes were significantly more likely to exhibit a reproductive abnormality than captive bred animals (p=0.003). In conclusion, contraception does not appear to be a risk factor for reproductive dysfunction in captive female great apes, however wild caught apes appear to be more susceptible to reproductive problems than captive born animals.

P24 Expression and localisation of the anti-inflammatory mediator Annexin A1 in normal human endometrium and in endometrial adenocarcinomas

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Introduction: Annexin A1 is a glucocorticoid-inducible effector protein which signals through the lipoxin receptor, FPR2, to inhibit inflammatory activation and promote resolution. This study investigated endometrial expression of Annexin A1 across the menstrual cycle and in endometrial adenocarcinoma, and examined modulation of Annexin A1 expression in an endometrial epithelial adenocarcinoma cell line in response to inflammatory stimuli.
Methods: Normal endometrium (N=60; proliferative, early/mid/late secretory, menstrual) and endometrial adenocarcinoma tissues (N=30; well, moderately and poorly differentiated) were obtained with Lothian Research Ethics Committee approval and informed patient consent. mRNA expression was determined using quantitative RT-PCR, and localisation of Annexin A1 together with FPR2 was examined by immunohistochemistry. Ishikawa endometrial epithelial cells were incubated with 100 nM phorbol 12-myristate 13-acetate (PMA) for 6 hours before harvesting.
Results and Discussion: Annexin A1 was found to be present throughout the menstrual cycle and is significantly up-regulated in both the mid-secretory phase and in carcinomas compared to normal proliferative endometrium. Immunohistochemistry revealed Annexin A1 is localised to glandular epithelial, endothelial and stromal immune cells with strong staining in the functional layer. In endometrial cancer expression of Annexin A1 was localised to neoplastic epithelial cells and the vasculature. Co-localisation of expression of Annexin A1 with the FPR2 receptor was observed in a population of cells of the endometrium and endometrial cancers suggesting autocrine/paracrine regulation of Annexin A1 action via FPR2. The Ishikawa endometrial epithelial cell line was also shown to express Annexin A1, and inflammatory stimulation with PMA resulted in significant up-regulation of expression of this anti-inflammatory mediator. These data demonstrate expression of the anti-inflammatory mediator Annexin A1 in normal endometrium and in endometrial cancer. Future studies will examine the intracellular localisation and activation state of Annexin A1 together with signalling through FPR2 in the context of endometrial pathologies.

P25 Characterization of reproductive physiology during the onset of premature ovarian failure in a mouse model

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Introduction: The reproductive lifespan of the ovary is limited by oocyte quantity. Double mutant mice (DM, *Tsyn*$^{F/F}$*Mgat1*$^{F/F}$:ZP3Cre) with oocyte-specific deletion of two glycosyltransferases are subfertile at 6-week. These mice undergo premature ovarian failure (POF) lacking functional ovaries by 3-month. We investigated estrus cycles, ovulation rate, and fertilization during POF onset in DM females.
Methods: To characterize estrous cycles, vaginal smears were analyzed daily from 4-week to 6-month. Mating was assessed from 6-week to 6-month by detection of a vaginal plug. Ovulation rate was determined in 6- and 9-week females at E0.5 post-mating. The zygotes were collected and cultured to late blastocyst in KSOM-AA at 37°C, 5% CO2.
Results and Discussion: At 6-weeks, there was no difference in the ovulation rate (8.2±0.4 control, 7.6±2.6 DM) or percentage of fertilized eggs (96±6.1 control, 89±13.7 DM) between controls (n=6) and mutants (n=5). The number of embryos that developed to the blastocyst stage also did not differ. Therefore the reduced fertility at 6-week is not due to defective ovulation or fertilization but due to defects at later stages. By 9-week, very few zygotes were collected from DM females (0.6±1.3, n=5) indicating that at this age the primary origin of infertility seems to be the complete lack of ovulated eggs. Despite the lack of eggs at 9-week, small cumulus cell clumps were present and regular mating took place until 6 months of age, demonstrating that DM females were still sexual receptive. Although the length of DM estrous cycles did not differ to controls and they still mated, DM females showed abnormal cycles with periods of persistent estrus indicative of acyclicity. In conclusion, modified oocyte glycoproteins in DM females do not affect ovulation rate at 6-week, but by 9-week eggs are no longer ovulated revealing that oocyte-glycoproteins are important regulators of ovarian function.

P26 Effects of siRNA-mediated inhibition of microsomal prostaglandin E synthase-1 in endometrial cells of the porcine uterus

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Introduction: Endometrium secretes elevated amounts of prostaglandin E2 (PGE2) into the uterine lumen and utero-ovarian circulation during maternal recognition of pregnancy in pigs. We have shown recently, that PGE2 stimulates expression of cyclooxygenase-2, PGE2 synthase (mPGES-1) and PGE2 receptor (EP2) in the porcine endometrium. Moreover, the endometrial EP2 expression is up-regulated on days 11-12 of pregnancy in comparison with days 11-12 of the oestrous cycle. The aim of this present study was to characterize the functional effect of mPGES-1 silencing in porcine endometrial stromal cells.
Methods: Specific short interfering RNA (siRNA) against *mPGES-1* were designed and cloned into an siRNA-expression vector (pSuper). Endometrial stromal cells isolated from uterus of gilts on day 11-12 of the oestrous cycle were cultured to 70% confluency and transfected by Lipofectamine with vectors expressing siRNA against *mPGES-1*, or with negative control vectors (containing either an siRNA of a gene not involved in PG synthesis or the empty pSuper vector). Expression of mPGES-1, EP2 and PGF synthase (PGFS) mRNA was determined by Real-time PCR. Secretion of PGE2 was examined by EIA.
Results and Discussion: Transfection of endometrial stromal cells with the mPGES-1/siRNA construct resulted in a reduction of mPGES-1 mRNA content by 50% ($p<0.001$) and PGE2 secretion by 40% in comparison with controls. Moreover, in mPGES-1-silenced cells of EP2 mRNA expression was decreased by 70% ($p<0.05$), whereas PGFS mRNA expression was unaltered. These results indicate an interdependence between the expression of mPGES-1 and EP2 in porcine endometrial cells. The presented data further confirm that PGE2 synthase and EP2 are involved in a PGE2 positive feedback loop in the porcine endometrium.

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Notes

P27 The action of tumor necrosis factor (TNF) on production and content of Arachidonic Acid metabolites in bovine immortalized endothelial corpus luteum cells

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Several distinct cell types, such as small and large luteal cells, vascular endothelial cells and pericytes, are distributed in the bovine corpus luteum (CL). So far, the interactions between the cells and its products: steroid and peptide hormones, prostaglandins (PGs), growth factors and cytokines in the regulation of CL function were studied. Cytokines may modify function of CL endothelial cells, enhancing $PGF_{2\alpha}$ action in CL. The aims are: (1) to establish and perform experimental in vitro model for bovine endothelial CL cells examination; (2) to study the effect of tumor necrosis factor □ (TNF) on production and secretion of Arachidonic Acid (AA) metabolites, especially leukotrienes (LTs) in bovine immortalized endothelial CL cells. The primary cultures of bovine CL endothelial cells (BEC) were immortalized by transfection of vector carrying a Simian virus 40 T-antigen (SV40 T-ag) sequence. Expression of SV40 T-ag gene in the cells was confirmed by RT-PCR. Selected immortalized line was cultured for next 50 passages without any sign of senescence. Additionally, immunofluorescence staining showed the presence of endothelial cell markers: VE-cadherin and von Willebrand factor. Immortalized BEC were stimulated by TNF (50 ng/ml) for 24 h. mRNA expression (real time RT-PCR), protein expression (western blotting) for LTC4 synthase, LTA4 hydrolase, PGE_2 and $PGF_{2\alpha}$ synthases and the level of endothelin-1 (EDN-1), LTs (B_4 and C_4) and PGs (E_2 and $F_{2\alpha}$) in the medium (EIA) were evaluated. TNF increased mRNA expression and protein expression each of studied factors. Although EDN-1, LTC_4 and $PGF_{2\alpha}$ secretion were elevated by TNF, the level of PGE_2 and LTB_4 not changed. Summarizing, EDN-1, PGs and LTs are produced and secreted by immortalized BEC showing proper secretory function of immortalized BEC. Immortalized BEC are sensitive on TNF action and this cytokine modulates expressions for enzymes responsible for production of AA metabolites and its levels.

P28 Role of phospholipase C zeta in human fertilization and infertility

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Introduction: During mammalian fertilization, intracellular calcium oscillations are triggered and sustained by the action of PLCzeta released by the sperm into the oocyte cytoplasm, leading to oocyte activation. Recently, PLCζ has been linked to cases of male factor infertility where the sperm is oocyte activation deficient. A point mutation of Histidine to Proline in the PLCzeta protein at position 398 (H398P) was identified in one infertile human male (Heytens et al., 2009: Human Reproduction 24:2417-28). Injection of a recombinant cRNA form of the mutant PLCzeta into mouse oocytes failed to trigger the normal pattern of calcium release. However, difficulties in generating active forms of recombinant PLCzeta protein have hampered attempts to analyse the enzymatic properties of wild type and mutant human PLCzeta.
Results and Discussion: Wild type and mutant forms of human PLCzeta were expressed in bacterial, yeast and mammalian expression systems. Good yields of pure PLCzeta protein were obtained using bacterial and yeast systems; assessment of activity using a sea urchin egg homogenate assay and injection into mouse oocytes showed the PLCzeta protein to be inactive, however these expression systems may offer a valuable route for generation of PLCzeta protein for structural studies. In contrast, PLCzeta protein expressed in human HEK293 cells triggered calcium oscillations when injected into mouse oocytes. Expression in HEK293 cells now offers the possibility of comparing the enzymatic properties of wild type and mutant forms of human PLCzeta as well as providing a system in which PLCzeta protein may be generated for therapeutic purposes, for example for treatment of infertility stemming from male factor oocyte activation deficiency forms of infertility.

Notes

P29 The use of Cefquinome as a treatment for Taylorella equigenitalis and the effect on fertility in the stallion

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Introduction: Contagious Equine Metritis is a sexually transmitted disease caused by the bacterium Taylorella equigenitalis (Te). In the Netherlands stallions have to be tested negative for Te before they are allowed to start in an artificial insemination breeding program. This test has to be repeated on an annual base and the stallions are not allowed to cover naturally. Some stallions are tested repeatedly positive on Te after treatment despite they don't cover naturally. The culture of the bacterium is the golden standard, however the PCR gives a much faster reliable result. The conventional treatment protocol does not give satisfactory results and chlorhexidin causes irritation of the penis. Therefore a new procedure was tested.
Methods: Stallions (n=8) repeatedly tested positive on Te after conventional treatment in culture and in PCR were treated as follows: one day treatment of the penis with chlorhexidin 2% and 75 mg cefquinome sulphate (Cobactan LC®, Intervet, Boxmeer, the Netherlands) as well as 3 consecutive days of intravenous injections with 2 mg/kg BW of cefquinome (Cobactan 4,5%®). After one and four weeks a swab sample from the fossa urethralis of the penis was submitted to both a culture- and PCR-test for Te. Seasonal fertility results were calcualted and compared to the results of non-Te-infected stallions. A T-test was used for statistics. The local ethical committee approved treatment with cefquinome.
Results and Discussion: Seven stallions were tested negative for Te in both culture and PCR, one week after treatment ($P<0.05$). After 4 weeks one stallion was tested positive on Te. Fertility seemed not to be affected after treatment ($P>0.05$). Conclusions: Te is sensitive to a treatment with cefquinome. However, it is likely that some stallions might need a longer washing period. There is a chance that the bacterium is present latent in the genital tract of the stallion and may have an intermittent excretion pattern.

P30 Cadmium induced male reproductive toxicity and protective effect of Melatonin in the Rat: duration dependent study

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Introduction: Cadmium induced Testicular toxicity has been assessed in an environmentally relevant realistic dosage schedule. The duration dependent study had been undertaken as humans are exposed to high dietary concentration of Cd in industrialized areas like Vadodara (INDIA).
Methods: Adult wistar rats were exposed to Cd(9mg/Kg BW) through drinking water with or without simultaneous administration(i.p.) of melatonin(10mg/Kg BW) at 1800hrs for 15, 30 and 60 days. All animals were maintained as per the guidelines of CPCSEA, India. On completion of the treatment schedule, animals were sacrificed and testes were used for estimation of metal load by ICP-AES and enzymic(SOD, CAT, GPx) and non enzymic (GSH, Vit. C) along with TBARS and activity levels of 3 β HSD and 17 β HSD using appropriate assay procedure. Histopathology of testis, epididymal sperm analysis and assay of serum titres T, E2 & Melatonin were also carried out.
Results and Discussion: Cadmium treatment resulted in significant increased in lipid peroxidation and decreased in antioxidant levels together with decreased activity of steroidogenic enzymes and lowered serum T and E2 levels. Significant, increased in testicular cadmium load and reduced serum melatonin level were also observed. Disruption in spermatogenesis and severe sperm abnormality were also observed as the features of relevance. Co-administration of melatonin showed significant protection against the Cd induced toxicity manifestations. On the whole the present study clearly shows a duration dependent toxic effects of Cd on male reproductive system in rats and, a potent action of melatonin in protecting the Cd induced toxic effects.

Notes

P31 Inhibition of mTOR induces cumulus expansion and meiotic maturation in mouse oocytes without gonadotropin stimulation

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The mammalian target of rapamycin (mTOR) signaling pathway functions as a central regulator of cell growth, proliferation, and survival. To investigate the role played by mTOR during meiotic resumption in mice, we cultured cumulus-oocyte complexes (COCs) in the presence of an mTOR inhibitor. mTOR expression was detected in the cumulus cells and was strong in the outer-layer cumulus cells in the GV stage. The COCs were cultured for 18 hours in a medium containing dbcAMP supplemented with the mTOR inhibitor PI-103 or rapamycin. We found that the cumulus expanded although the nuclei had been arrested in the GV stage. These oocytes were then transferred to fresh maturation medium containing FSH and an mTOR inhibitor and cultured for 8 hours. We found that the development of the first polar body was premature in oocytes that had been treated with the mTOR inhibitor. This result suggests that mTOR inhibition induces early progression of the nuclear stage. Further, when GV-stage oocytes were cultured for 18 hours in maturation medium lacking FSH but containing the mTOR inhibitor PI-103 or rapamycin, the cumulus expanded and the first polar body successfully developed. These MII oocytes were subjected to in vitro fertilization, and the fertilized embryos developed into blastocysts. In addition, we found that the expression of hyaluronan synthase (HAS) in the cumulus cells increased on treatment with an mTOR inhibitor. In conclusion, we determined that mTOR is involved in cumulus expansion and meiotic maturation in mice. In the presence of an mTOR inhibitor, cumulus expansion occurred and meiotic maturation progressed without gonadotropin stimulation. Thus, a similar mechanism may operate in vivo, with mTOR inhibition in cumulus cells serving as a trigger for gonadotropin-induced meiotic maturation.

P32 Prostaglandin E2 Partially Mediates Gonadotropin-Dependant Bovine Oocyte Maturation

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Introduction: Gonadotropins (Gn) are suggested to be necessary for oocyte maturation and cumulus cell expansion in mammals. In rodents, prostaglandin E_2 (PGE_2) is an additional mediator of oocyte maturation. However, in cattle, the role of PGs in oocyte maturation remains unelucidated despite key components of PG synthesis, cyclooxygenase (COX) and PGE synthase enzymes being expressed in cumulus cells.
Methods: Experiment 1. Bovine oocytes were cultured in serum-free maturation media supplemented with or without increasing concentrations (500 -2000 pg/ml) of PGE_2, or $PGF_{2\alpha}$ or both, in the presence or absence of Gn (FSH and LH; 10 µg/ml). Experiment 2. Oocytes were cultured in Gn-containing media supplemented with or without specific COX2 inhibitor (NS398; 10 µM) or EP2 receptor antagonist (AH6809; 40 µM). Concentration of PGE was measured in the spent media by radioimmunoassay. In both experiments cumulus cell expansion and oocyte nuclear maturation (MII stage) were assessed after 24h.
Results and discussion: Prostaglandin E_2 had no effect on oocyte maturation in the presence of Gn stimulation. However, in the absence of Gn, PGE_2 stimulated partial expansion of cumulus cells at all concentrations, and increased the number of oocytes at metaphase-II when supplemented at 500 pg/ml. In contrast, $PGF_{2\alpha}$ had no effect on oocyte nuclear maturation, and higher levels of $PGF_{2\alpha}$ decreased cumulus cell expansion both in the presence and absence of Gn and/or PGE_2. Both NS398 and AH6809 significantly decreased oocyte nuclear maturation compared to the control. NS398 significantly decreased the level of PGE production. These data provide evidence to support the view that PGE_2 (via the EP2 receptor) can exert similar effects to gonadotropins on bovine oocyte maturation. Furthermore they show that these gonadotropin effects are dependant, at least in part, on PGE_2 synthesis and action.

Notes

P33 Liposome-mediated mRNA transfection of mammalian oocytes

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Introduction: The transfection of mouse oocytes using liposomes containing foreign DNA was already published (1). The main goal of this study is to evolve a technique which enables to insert exogenous mRNA into the mammalian oocyte without a need of expensive microinjection equipment. Therefore, we focused on transfection of in *vitro* synthetized capped mRNA with poly(A)tail into immature mouse and pig oocytes to follow meiotic maturation by chromatin visualization.
Methods: We performed liposome-mediated transfection using commercial available kits from two different suppliers. Oocytes from both species were transfected with histone H2B mRNA tagged with mRNA of fluorescent protein (mCherry) whose protein product decorates chromatin and enables direct evaluation of oocyte maturation stage. Since zona pellucida (ZP) in mammals provide the protection of the oocyte from foreign DNA acceptance, it must be permeabilised or removed to allow transfection. Hence, enzymatic treatment of 0.5% pronase and Tyrode´s buffer was used to remove ZP from porcine and mouse oocytes, respectively.
Results and Discussion: When ZP was completely removed, we were able to transfect immature oocytes from both species, whereas in presence of weakened ZP, the transfection was almost undetectable. It should be mentioned that the way how zona pellucida is removed represents crucial step in this technique. We observed good transfection efficiency (80% in some cases) for both species and our preliminary results showed that approx. 85% of mouse oocytes and 75% of porcine oocytes reached the metaphase II stage. This indicates that liposome-mediated transfection of mRNA is feasible way how to insert exogenous mRNA into mammalian oocytes.

P34 Impact of follicle environment and assisted reproductive technology on indices of bovine oocyte developmental competence

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Introduction: The follicular environment and assisted reproduction technologies have been shown to influence oocyte developmental competence (quality) in a number of species. This study investigated the link between cellular, molecular and metabolic indices of follicle development and oocyte quality in bovine oocytes obtained following in vitro maturation (IVM) and controlled ovarian stimulation (COS).
Methods: Cumulus oocyte complexes (COCs) were collected from follicles dissected from abattoir-derived ovaries (G1). COCs were matured individually for 18hrs in 10µl drops of IVM media containing oFSH (0.001IU/ml) and oLH (0.0002IU/ml). Alternatively, COCs were collected from heifers (n=6/group) which had been down regulated with Buserelin prior to COS by i.v. infusion of: oFSH (50µg/h for 120h, G2); or oFSH+oLH (oFSH 50µg/h for 72h, followed by oLH 25µg/h for 72-120h, G3); animals were injected with hCG (3000IU, i.m.) at 120h and OPU performed 16-20h later. Following denudation, individual G1-3 oocytes were cultured for 6hrs in 1µl of modified IVM media to measure oocyte amino acid profiles (AAP) by HPLC. Follicle diameter, dominance, cumulus morphology and oocyte maturity were recorded.
Results and Discussion: COCs from a range of follicle sizes (3-20mm) were analysed. Cumulus coverage was not significantly different between groups ($P>0.05$) but COCs from G2 (70%, n=50) and G3 (80.8%, n=52) had a significantly ($P<0.05$) greater cumulus expansion than G1 (51.7%, n=118). The maturation rate for G3 oocytes (66.7%; n=51) was significantly ($P<0.05$) lower than G1 (80.8%; n=120). Aspartic acid and Leucine metabolism was different ($P<0.05$) between immature and MII oocytes. The three treatments significantly ($P<0.05$) affected overall amino acid turnover by MII oocytes and altered the metabolism of Glutamic Acid, Serine, Glutamine, Glycine, Threonine, Arginine, Tryptophan and Methionine. The data suggests a strong link between amino acid metabolism, follicular environment and oocyte developmental competence. (This work was supported by the Infertility Research Trust).

P35 Ovine oocytes vitrified at germinal vesicle stage as cytoplast recipients for Somatic cell nuclear transfer (SCNT)

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The development of SCNT embryos produced using vitrified oocytes as cytoplast recipients has been reported in cattle and pigs; but not in sheep. This study investigated the effects of vitrification on spindle and chromosome configuration, maturation promoting factor (MPF) and mitogen activated protein kinase (MAPK) activities following IVM. The developmental competence of ovine oocytes vitrified at the germinal vesicle stage, matured and used as cytoplast recipients for SCNT was then determined. Vitrified/thawed oocytes were matured *in vitro* (Moawad *et al.* 2010). Spindle and chromatin configuration were examined, MPF/MAPK activities measured and SCNT, using foetal fibroblasts as nuclear donors carried out as previously described (Lee and Campbell 2006). The proportion of oocytes with normal spindle and chromatin morphology was lower in vitrified group as compared to control (50.0% v 70.4%); however, the proportion of oocytes with abnormal spindle was higher in vitrified oocytes than control (20.8% v 7.4%, P=0.067), both MPF and MAPK activities were decreased in vitrified oocytes but this was not significant. Following SCNT, very high enucleation (~ 99.0%) and fusion (~ 98.0%) rates were achieved in both groups. Cleavage at 24 hpa (31.0% v 55.1%) and 48 hpa (48.0% v 85.0%) was significantly lower (P< 0.05) in vitrified oocytes as compared to control. However, no significant differences were observed between two groups in terms of morula (38.0% v 46.7%), and blastocyst (13.0% v 23.4%) development. Interestingly, the percentage of cleaved embryos that developed to blastocysts was similar in both groups (27.0%). The same trend was noticed in hatched blastocysts (7.0% v 10.3%), total cell numbers (90.3 v 97.6), number of apoptotic nuclei (13.1 v 13.2), and proportion of diploid embryos (60.0% v 75.0 %). This study demonstrates for the first time that vitrified/thawed ovine oocytes can be used successfully as recipient cytoplasts for SCNT.

P36 Evidence that MCAK-dependent error correction is not nessesary for preventing aneuploidy in oocytes

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Chromosome segregation errors in mammalian oocytes lead to aneuploid eggs which are developmentally compromised. In somatic cells Mitotic Centromere Associated Kinesin (Kif2C/MCAK) detaches incorrect microtubule-kinetochore interactions in mitosis, and depletion or inhibition of MCAK causes chromosome segregation errors. Since in oocyte meiosis I (MI), unlike mitosis, stable kinetochore-spindle interactions are thought to be formed late in prometaphase, after chromosomes have been correctly aligned, it is unknown whether the MCAK-pathway should be relevant. Intriguingly, however, recent micro-array analyses revealed that MCAK transcripts are reduced by half in oocytes from older mice, which are predisposed to aneuploidy similar to aging human eggs. This study therefore investigates the role MCAK plays in mammalian oocytes, to determine if a reduction in MCAK in otherwise healthy oocytes from young mice can lead to chromosome misalignment and aneuploidy. Using immunofluorescence we show that MCAK is recruited to centromeres, kinetochores, and chromosome arms in mid-MI. Injection of MCAK morpholino removes centromeric MCAK and causes chromosome misalignment in MI. MCAK dominant negative causes a similar extent of misalignment. However, the majority of oocytes complete MI and the resulting eggs retain the correct chromosome number. Simultaneous depletion of MCAK and expression of the dominant-negative also failed to induce aneuploidy. MCAK-depleted oocytes can even recover from an experimentally-induced mal-orientation of chromosomes in mid-MI to produce eggs with the correct number of chromosomes. Thus, MCAK contributes to chromosome alignment in MI, but is not normally necessary for preventing aneuploidy. Whilst other correction mechanisms may function in mammalian MI, we suggest a model in which late establishment of kinetochore microtubules reduces the likelihood of incorrect MT-kinetochore interactions, whereby mammalian oocytes normally bypass the requirement for error correction.

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P37 Activity 11beta-hydroxysteroid dehydrogenase (11betaHSD) enzymes during the maturation of mouse cumulus oocytes complexes

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Introduction: The maturation of oocytes from prophase I to metaphase II (MII) is a tightly regulated process. We have previously reported that the net oxidation of the active glucocorticoid cortisol to inert cortisone by 11beta-hydroxysteroid dehydrogenase (11betaHSD) enzymes increases with maturation of porcine oocytes. While 11betaHSD1 acts as both an 11-ketosteroid reductase and 11beta-dehydrogenase, 11betaHSD2 acts exclusively as a high affinity dehydrogenase to inactivate glucocorticoids. Recently we demonstrated increased expression of the mRNAs encoding 11betaHSD2 and altered subcellular localisation of both 11betaHSD proteins during the maturation of mouse oocytes. The aim of this study was to characterise the activity 11betaHSD isoenzymes in mouse cumulus oocytes complexes (COCs) during maturation.
Methods: COCs were recovered in media from gonandotrophin-primed MF1 mice. Enzyme activities were assessed over 24 hours in serum-free KSOM using radiometric conversion assays with 100nM [3H]-cortisol and 100nM [3H]-cortisone as substrates.
Results and Discussion: In IBMX (200uM) arrested COCs 11beta-dehydrogenase activity was 287±22 fmol/oocyte.24h (mean±se; n=9) and this enzymatic inactivation of cortisol was inhibited by addition of the 11betaHSD inhibitor, carbenoxolone (10µM; 39±5 fmol/COC.24h; n=4; *P*<0.001). When the COCs were removed from IBMX the enzyme activity was doubled (559±37 fmol/COC.24h, n=8; *P*<0.001). Furthermore addition of EGF (20ng/mL) increased the net 11beta-dehydrogenase activity of both arrested and released COCs (799±12 and 1132±9 fmol/COC.24h, respectively, n=4; *P*<0.001). There was no detectable 11-ketosteroid reductase activity (n=2). These data are the first demonstration of 11betaHSD activity in mouse COCs and suggest that glucocorticoids are inactivated (rather than regenerated) by 11betaHSD enzymes. Furthermore, this enzymatic inactivation of glucocorticoids is increased in mouse COCs during spontaneous oocyte maturation and following the addition of the meiotic induction factor, EGF.

P38 The effects of androgens on the developmental capacity of bovine oocytes and embryo production in vitro

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Androgens are readily available in follicular fluid (FF), exerting auto- and paracrine actions upon mural and COC-linked granulosa cells, and the oocytes contained in pre-ovulatory ovarian follicles. Currently, testosterone (T) and androstenedione (A4) are not included in chemically defined media used for in vitro embryo production (IVP). This study examined the effects of the androgens added to in vitro maturation (IVM) media prepared for bovine IVP. Oil-free IVM was employed to avoid a hormone “sink” improving steroid bioavailability in the androgen-supplemented media. We also compared glassware with the routinely used plastic culture wells to examine differences in the material-steroid interactions. Oocytes were matured for 22 h in IVM media supplemented with one of two concentrations of T or A4 (T: 22.2 and 42.6 ng/mL, A4: 562.5 and 337.5 ng/mL). Doses are based upon previously reported FF samples from pre-ovulatory bovine follicles (mean ± 25%). Control and supplemented IVM media were then incubated, with or without COCs, in plastic or glassware. Embryo development was monitored from cleavage until day 8 post-insemination (blastocyst stage). Addition of A4 resulted in detectable increases in T levels. The reverse trend was observed after the addition of T. Elevated T concentrations were most prominent after A4 addition to media containing COCs. The mean cleavage rate was lower (P<0.05) after incubation in glass compared to plastic IVM dishes containing control media. Maturation with A4 resulted in normal cleavage rates (P>0.05) regardless of material. In the present experiment, no differences (P>0.05) were noted in blastocyst formation rates. We concluded that IVM in glassware resulted in lower cleavage rates, a decrease that was prevented by addition of physiological levels of A4. Neither the material nor addition of androgens appeared to alter embryo development to the blastocyst stage in vitro.

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Notes

P39 FSH/LH induced expression of EGF-like factors in pig granulosa cells and cumulus-oocyte complexes cultured in vitro

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Introduction: The effect of LH in rodent preovulatory follicle is mediated by production of EGF-like peptides, amphiregulin (AREG), epiregulin (EREG) and betacellulin (BTC) by mural granulosa cells (MGC) and consequently also by cumulus cells. These peptides then regulate cumulus expansion and resumption of oocyte meiosis. The objective of this work was to assess whether similar mechanisms regulate maturation of pig cumulus-oocyte complexes (COCs) in vitro.
Methods: COCs and MGC were harvested from follicles of immature gilts and cultured in medium M-199 with 5% of fetal calf serum, human recombinant FSH, LH (Serono) or both for 0, 1, 2, 3, 4, 8, 16, 24 and 28 h. At the end of culture, the total RNA was extracted and a relative abundance of EREG, AREG and BTC mRNA in the samples was assessed by a real time RT-PCR by use of the RotorGene 3000 cycler. The results for individual target genes were normalized according to their relative concentration to the internal standard (HPRT).
Results and Discussion: FSH and LH significantly stimulated expression of AREG and EREG, but not BTC in MGC at 3 and 4 h of culture; addition of both FSH and LH had an additive or even synergic effect. LH moderately (4 fold) increased expression of AREG and EREG in COCs whereas FSH stimulated 10 – 20 fold increase at 3 and 4 h of culture. Combination of FSH and LH did not increase EREG and AREG expression over the level found in FSH-stimulated COCs. No significant increase in AREG, EREG and BTC expression was found beyond 4 h of culture in both MGC and COCs, regardless of the type of stimulation. These data indicate that FSH/LH induced maturation of pig oocytes is mediated by production of AREG and EREG in granulosa and cumulus cells. Supported by GACR No. 523/08/0111.

P40 The strategy for shipment of cryopreserved mouse oocytes at -80ºC

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Introduction: The world wide, federation of international mouse resources (FIMRe) is aiming to coordinate the generation of mutations in up to 20,000 mouse strains and promote the cryopreservation and sharing of these genetic resources between laboratory and such facility. Those mouse mutants are to be cryopreserved as frozen oocytes/sperm/embryos at -196ºC in the facilities. If we can transport them with dry ice at -79ºC between laboratory and facility, it is easy and inexpensive. We looked at the question of whether oocytes frozen to -196ºC can survive at -80ºC.
Methods: The mouse metaphase II oocytes were cryopreserved in EAFS10/10 solution with vitrification procedure developed by Dr. M. Kasai's group at Kochi University, Japan. The extracellular ice formation in the medium was induced by relative slow cooling (187 or 294ºC/min) to -196 or -80ºC and the oocytes were held at -80ºC for 0-3 months. The survivals were determined after thawing. All procedures were approved by the University of Tennessee IACUC protocol 911-607.
Results and Discussion: Even if the holding time at -80ºC reached to one month, the survival by highest warming (2950 oC/min) was still high (73%). However, if the warming rates were low (139 or 476ºC/min), the survival was low (5-14%). The results indicate that oocyte can be shipped at -79ºC with dry ice without much loss in their survivals, provided that they are then returned to -196ºC.

Notes

P41 Exposing germinal vesicle stage oocytes to elevated temperature affects gene expression at the MII stage; in-vivo and in-vitro models in bovine

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The ovarian pool of follicles is highly susceptible to high ambient temperature. However, the effect on follicle-enclosed oocytes is not clear. The objectives of this study were to examine the effects of elevated temperature on germinal vesicle (GV)-stage oocytes and to determine whether *in-vitro* heat shock has a deleterious effect similar to that of environmental thermal stress. Ovaries were collected from slaughtered Holstein cows through both the hot (June-Nov) and cold (Dec-Apr) seasons. Cumulus oocyte complexes (COCs) were aspirated from 3- to 8-mm follicles and subjected to *in-vitro* embryonic production. For the *in-vitro* model, COCs were collected in the cold season and pre-matured with the meiosis inhibitor IBMX (75µM) for 16h at 38.5 or 41.2ºC. For both *in-vivo* and *in-vitro* models, expression of *C-MOS*, *GDF9*, *GAPDH*, and *POU5F1* genes was examined at the MII stage by real-time PCR, using *18S rRNA* as a reference gene. Cleavage rate and blastocyst development (at 42h and 7-8d post fertilization, respectively) were higher during the cold season (90.2±0.87% and 24±0.97%, respectively) than the hot season (78.2±2.2% and 9±1.02%, respectively; P<0.05). Pre-maturation with IBMX successfully blocked germinal vesicle breakdown without compromising oocyte competence to undergo maturation. The proportion of fertilized oocytes that cleaved and developed to the blastocyst stage, at 38.5ºC, was 85±3.1% and 20±0.7%, respectively. Similar to the *in-vivo* model, pre-maturation of GV-stage oocytes with IBMX at 41.2ºC reduced cleavage rate and the proportion of developing blastocysts (75±1.2% and 5.6±0.9%, respectively, *P*<0.05). Furthermore, in both models, exposing GV-stage oocytes to elevated temperature further reduced *(P<0.05) C-MOS, GDF9, GAPDH and POU5F1* gene expression at the MII stage. It appears that the *in-vitro* model can be used to examine the effects of environmental heat stress on oocyte gene expression. Whether this is also true for further embryonic developmental stages is not yet known.

P42 Relative mRNA expression of several candidate competence genes in sheep oocytes in relation with oocyte quality

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Introduction: It is well known that several factors such as follicle size and health, donor age, season and nutrition may affect oocyte developmental competence. In the present experiment our aim was to identify the relative transcript expression (RE) of five genes associated with oocyte quality (Tornet H. etal. 2008). Adult and prepubertal sheep oocytes were used as models of "high" and "low" quality respectively.
Methods: Cumulus oocyte complexes (COCs) from adult and prepubertal sheep were isolated from ovaries collected from a slaughterhouse. Morphologically selected COCs from ewes and lambs were matured for 24 hours in vitro in an enriched TCM199 medium. Samples destined for gene-expression analysis were taken before (T0) and after (TiVM) in vitro maturation by denuding COCs by gentle pipetting and stored at -80°C in 100 µL Trizol until use. Total RNA was extracted from groups of 15 oocytes (4 replicates per condition). Reverse transcription and real-time PCR analysis using SYBR Green was performed. RE was calculated using 18S as the internal control gene.
Results and Discussion: RE of ornithine decarboxylase (ODC) and elongation factor A1 (EEF1A1) differed between lambs and ewes in mature oocytes. Cytochrome c oxidase subunit (COX1), calcium-binding protein (S100A10) and EEF1A1were differently expressed in immature as compared to mature oocytes whatever the age of the animals. No difference in mRNA expression patterns was observed for nuclear autoantigenic sperm protein (NASP) gene. In conclusion, RE of some genes correlated with maturation stage and oocyte quality in prepubertal and adult sheep oocytes. Significant differences were detected in RE pattern between adult and prepubertal matured oocytes.

Notes

P43 The role of membrane trafficking proteins during cell division in mouse oocytes

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Mouse oocytes undergo two successive meiotic divisions to give rise to one large functional oocyte and two small polar bodies. These divisions are crucial as anomalies in this process would preclude normal fertilization and regular development of the embryo. How this asymmetric cytokinesis is controlled is still poorly understood. The purpose of my study is to focus on the cellular events occurring during cell division (cytokinesis) in mouse oocytes, and in particular on the role of specific membrane trafficking proteins which have been found to play a role during cell division in other model organisms. We have shown that phosphatidylinositol transfer protein β (PITPβ), a membrane trafficking protein which localises to the Golgi in somatic cells, does not localise to the Golgi in mouse oocytes, instead it is found in the early endosome compartment. Overexpression of PITPβ in oocytes at the metaphaseII stage causes an abnormal accumulation of early endosomes compared to the controls. In addition, we have investigated the role of another membrane trafficking protein called Rab11, which is a small GTPase binding protein. We have found that this protein localises strongly at the cleavage furrow of oocytes undergoing the first meiotic division. In addition, more than half oocytes injected with a Rab11S25N dominant-negative mutant remain arrested at metaphasel and do not extrude a polar body. Further studies are required in order to determine the relationship between PITPβ, Rab11 and other membrane trafficking proteins during the meiotic cell divisions in mouse oocytes.

P44 Cytokines in follicular fluid: a potential tool to define oocyte maturity in intracytoplasmic sperm injection?

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Introduction: Current embryological practice identifies oocyte maturity at the time of collection by the presence of a polar body; however maturity assessment in the presence of cumulus cells is unreliable/subjective. Cytokines play a central role in oogenesis and folliculogenesis, and thus may act as biomarkers for identifying oocyte maturity prior to cumulus stripping. This study aimed to identify follicular fluid cytokine profiles which relate to oocyte maturity and fertilisation potential.
Materials and Methods: Ethical approval was gained from the Leeds East Research Ethics Committee, all participants gave informed written consent. Patients (n=64) undergoing ovarian stimulation with Menopur Daily (mean starting dose 347IU, range 150-750IU) were recruited. Follicular fluid from individual follicles was collected at the time of oocyte retrieval. Oocytes were classified following cumulus stripping, and mature oocytes fertilised by ICSI. Fertilisation outcome/maturity status for individual oocytes was related to source follicle cytokine profiles. 50 cytokines were quantified by fluid-phase multiplex immunoassay and levels standardised against protein and corrected for blood contamination. Data were analysed using Mann Whitney U tests.
Results and Discussion: Several cytokines differentiated between degrees of oocyte maturity (GV, metaphase I and metaphase II). Within the metaphase II category, several cytokines further differentiated between oocytes which fertilised and those which did not (eotaxin, CTACK, MCP-3, M-CSF, βNGF, TRAIL and SDF1α, $p \leq 0.01$; VEGF, GRO-α $p \leq 0.05$). This suggests that follicular fluid cytokine profiles may be of value in differentiating maturity/fertilisation potential within metaphase II oocyte pools, adding an objective, quantitative aspect to the assessment of nuclear and cytoplasmic maturity. These findings may also facilitate the identification of oocytes which could benefit from extended culture to reach full maturity in a manner similar to in vitro maturation, increasing the number of oocytes available for fertilisation. (The authors are indebted to the Infertility Research Trust and Leeds Reproductive Bioscience for funding).

Notes

P45 Why do proven methods of hormonal induction of ovulation consistently fail in a marsupial - the common Australian brushtail possum Trichosurus vulpecula?

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Introduction: The brushtail possum, introduced into New Zealand in the 1850's, is now a major agriculture and conservation pest. Current research aimed at reducing reproductive success in possums as a means of population control, requires reliable methods for synchronising oestrus and ovulation. The objective of these series of experiments was to adapt proven hormonal synchronisation protocols that are routinely used in farm species, for brushtail possums.
Methods: Wild-caught brushtail possums (N=330) were subjected to various treatments to synchronise ovulation in the breeding season, or to induce ovulation in the non-breeding season. Treatments included removal of suckling pouch young (RPY), extended exposure to progestagens to block ovulation, oestradiol injection to eliminate large antral follicles at the time of progestagen administration, treatment with FSH and prolactin following progestagen withdrawal to promote antral follicle development and treatment with oestradiol, GnRH, LH or hCG to induce ovulation of existing preovulatory (≥5mm diameter) follicles. Progressive development of preovulatory follicles and time of ovulation were determined by repeated laparoscopic examination of ovaries at frequent intervals.
Results and Discussion: Following RPY alone, 58% of animals ovulated 6-18 days later. High-dose progestagen implants blocked ovulation, but subsequent ovulation (7-18 days post-P4 withdrawal) was not synchronous and not all animals ovulated. FSH treatment promoted antral follicle development, but did not improve the incidence or synchrony ovulation. The administration of bolus doses of oestradiol, ovine LH, hCG or GnRH all failed to induce ovulation of preovulatory follicles, although repeated injections of GnRH induced rupture of 25% of these follicles within 48h. Known differences in possum follicle development include expression of LH receptor in granulosa cells much earlier and morphological differences in the cumulus cell phenotype (single layer of granulosa cells). We suggest that failure of these hormone treatments, reflect other differences in hormonal control of preovulatory follicle development in possums.
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P46 Dysregulation of pathways controlling hormone production and vascular development and function in the corpora lutea of short cycles following the male effect

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With an outcry for "clean, green and ethical" animal production, and an ever-increasing, consumer-driven global demand for organic produce, a requirement for hormone-free farming has never been so important. The male (or ram) effect, is a well established, hormone-free method of mating ewes outside the breeding season, however, for unknown reasons, luteal failure occurs and a short-cycle is observed in up to 50% of the anoestrus flock. Ideally, the elimination of this short cycle, which results in two peaks of lambing, would improve the efficiency of this technique. Here, we aimed to determine the cellular and molecular events associated with the short-cycle, a problem which remains largely unexplored. We stimulated a flock of confirmed-anoestrus, Ile de France ewes during the seasonal anoestrus (June, 2009) and collected ovaries three and seven days following the ram effect to isolate ovulatory follicles and corpora lutea (CL). Daily venous samples were collected and ovarian venous samples collected at ovariectomy. Hormonal profiling allowed identification of a group of ewes (day 7) with short cycles. Ewes with short cycles (day 7) had significantly lower jugular and ovarian venous progesterone concentrations, however, circulating prostaglandin F metabolite was not different. Real-time RT PCR analysis revealed the dysregulation of a number of pathways essential for luteal function. Short cycles CLs had significantly lower expression of genes essential for the synthesis of progesterone (*Star, Cyp11a1, Hsd3b*) and factors necessary for the development/function of the luteal vasculature (*Vegfa, Vegfr2, Lyve1*). This study reveals for the first time, the dysregulation of pathways critical for normal luteal function, and provides the first significant insight into the mechanisms responsible for the failure of the CL following the male effect during anoestrus.

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Notes

P47 Exploring TCam-2 cells as a model for the study of human fetal germ cell development

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Introduction: Progress in dissecting the molecular mechanisms regulating early germ cell (GC) development has been impaired by the lack of a reproducible *in vitro* culture system enabling the expansion, manipulation and trans-meiotic differentiation of GCs. The TCam-2 line is derived from a human GC tumour and has been proposed as a potential model in which to study human fetal GC development. This study aims to determine the phenotypic similarities between TCam-2 and human fetal GCs and establish their potential as a model for early GC development.
Methods: TCam-2 cells were maintained in RPMI1640 with 10% FCS, 2mM L-Glutamine. RT-PCR, western blotting and immunofluorescence analyses were performed using standard methods.
Results and Discussion: TCam-2 cells expressed a range of markers of primordial (*OCT4, NANOS3*) and meiotic (*SYCP3, STAG3, DMC1*) human fetal GCs. DAZL, a key regulator of GC development and meiotic entry, was undetectable at both mRNA and protein level. Consistent with the requirement for DAZL in regulating the translation of VASA in murine GCs, TCam-2 cells expressed *VASA* mRNA but not protein. Conversely, SYCP3 protein was readily detectable (and forms extensive networks of cytoplasmic fibres); indicating that the requirement for DAZL in promoting the translation of this protein in mice is not conserved to humans. Treatment with Retinoic Acid (RA; 1μM, 8 hours) failed to promote expression of the meiosis marker *STRA8*, but induced a 2-fold increase in the expression of *RARβ* (a known RA-responsive gene). As DAZL is required for GCs to enter meiosis in response to RA, restoration of DAZL expression in TCam-2 cells may facilitate their meiotic differentiation. These data demonstrate that TCam-2 cells share significant phenotypic similarities with human fetal GCs, and indicate they may have considerable utility in dissecting the molecular basis of human GC development.

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P48 Maternal cigarette smoking and its effects on follicle and somatic cell development

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Introduction: Women exposed prenatally to maternal cigarette smoke chemicals have reduced fecundity. Lutterodt et al. (Human Reproduction 2009 24:2558) recently showed that in-utero exposure to cigarette smoke during the first trimester reduced human fetal ovarian somatic cell numbers. We hypothesised that this effect would impair primordial follicle formation. To test this hypothesis we conducted an exploratory study counting the numbers of follicles and oocytes and quantifying expression of key proteins in late second trimester human fetal ovaries.
Methods: Gonads and plasma were collected from 10 female fetuses (5/group, matched for age and size) from electively-terminated, normally-progressing pregnancies (17-20 weeks, REC 04/S0802/21). Fetal plasma cotinine concentrations were determined to confirm cigarette exposure. Naked oocytes and primordial follicles were manually counted in whole ovarian cross-sections. NALP5, YBOX2, ZP2, CYP19, OCT3/4 and VASA immunostaining was quantified manually and using Volocity®. Data was analyzed with respect to gestational age and maternal smoking status (ANOVA or Wilcoxon test, as appropriate).
Results and Discussion: Total numbers of naked oocytes and primordial follicles, normalised by ovarian area, were not significantly different between exposed and control groups. However, primordial follicles normalised to ovary area and weight tended to be reduced in smoke-exposed fetuses (1.39-fold and 1.68-fold respectively) as was the primordial follicle:naked oocyte ratio (1.27-fold lower). YBOX2 positive primordial follicle numbers were similar between groups (p=0.983) while ZP2 positive primordial follicle numbers tended to be reduced in smokers (p=0.073). CYP19 correlated negatively with numbers of primordial follicles (r=0.700, p=0.003) while the proportion of ovarian area positive for CYP19 immunostaining was significantly increased in smoke-exposed fetuses (2.55-fold, p=0.034). In conclusion, our data suggest some impairment of primordial follicle formation by in-utero exposure to maternal cigarette smoking. However, further investigation with a larger population size is required.

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Notes

P49 Investigations into ovarian follicle development and apoptosis in a mouse model of premature ovarian failure

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Background: A mouse model of follicular premature ovarian failure, called the double mutant (DM), has been generated. DM females produce oocytes that lack two glycosyltransferase genes due to oocyte-specific deletion using Cre LoxP recombination technology. The floxed genes are deleted by a ZP3 *Cre* transgene which is expressed exclusively in the oocyte from the primary stage of development. Therefore, deletion occurs after primordial follicles have entered the growing pool and thus all stages prior to this remain unaltered. The DM is infertile after 3 months due to a reduced number of developing follicles in the ovary, despite having a normal number of primordial follicles. The aims of this investigation were therefore to determine if follicle development has altered in double mutant ovaries and to determine if follicles are being removed from the DM ovary by apoptosis.
Methods: Three 7 week old experimental mice (*T-syn*$^{F/F}$*Mgat*$^{F/F}$:ZP3*Cre*) and three age matched controls (*T-syn*$^{F/F}$*Mgat*$^{F/F}$) were selected. Ovaries were collected, fixed, embedded and sectioned at 5μm. Follicle counts were used to determine the number of primary, secondary, preantral and antral follicles in DM and control ovaries. Immunohistochemistry for Ki67 and the TUNEL assay were used to identify proliferation and apoptosis in granulosa cells, respectively.
Results and Discussion: DM ovaries have a normal number of primary follicles, but an average of 70% fewer secondary, preantral and antral follicles than controls. There is no significant difference between the growth and removal of control follicles to DM follicles that do progress past the primary stage. Thus, it is likely that the reduced number of developing follicles in the 3-month DM ovary is due to failure of the majority of follicles to progress past the primary stage of development. Therefore, oocyte glycoproteins play an important role in the progression of follicular development past the primary stage.

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P50 SPARC expression is regulated by TGFB1 during the bovine follicle-luteal transition

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Introduction: SPARC (secreted protein, acidic, cysteine-rich) is a matri-cellular protein, the levels of which increase during follicle-luteal transition. We found previously that LH has no effect on SPARC protein expression in luteinised granulosa cells. Studies with non-ovarian cells indicate that SPARC is up-regulated by transforming growth factor, beta 1(TGFB1) and it is known that TGFB1 is expressed in the bovine follicle. Here we tested the hypothesis that TGFB1 increases SPARC protein expression in bovine luteinised granulosa cells, and that this effect is mediated primarily via the receptor TGFBR1.
Methods: Granulosa cells were harvested from bovine large-antral follicles and seeded at 400,000 viable cells/well, containing DMEM-F12 medium with 1% FCS, in 12-well plates. Cells were treated with 0, 1 or 10ng/ml TGFB1 in the presence or absence of 2μM SB505124 (a TGFB1R inhibitor). Cells were harvested at 48, 96 and 144h for the determination of SPARC protein expression by Western blotting. SPARC levels were determined by densitometry and normalised to the loading control histone H3.
Results and Discussion: Cell number, determined by protein concentration, increased ($P<0.001$) with TGFB1. SB505124 reduced ($P=0.014$) total protein at 96 and 144h but not at 48h culture. The presence of SPARC protein was low or absent at 48h in control cells, but thereafter increased ($P<0.001$) 3-fold and reached a maximum at 144h. TGFB1 increased ($P<0.001$) SPARC protein expression in a dose-dependent manner at all time points. In the absence of TGFB1, SB505124 reduced ($P<0.001$) SPARC protein expression by 2- and 4-fold at 96 and 144h. Moreover, SB505124 reduced TGFB1 stimulated SPARC expression by between 2- and 3-fold ($P<0.001$). In conclusion, TGFB1 increased SPARC protein expression via TGFBR1 in luteinised bovine granulosa cells.

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P51 Levels of BMP-6 mRNA in goat ovarian follicles and in vitro effects of BMP-6 on secondary follicle development

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This study aims to verify the level of BMP-6 mRNA in follicles of different stages of development and to investigate the influence of BMP-6 on the growth of secondary follicles cultured for 6 days. Therefore, goat primordial, primary and secondary follicles, as well as COCs and granulosa / theca cells from small and large antral follicles were obtained and the mRNA levels of BMP-6 were quantified by PCR in real time. Expression of BMP-6 protein in goat follicles was demonstrated by immunohistochemistry. The influence of BMP-6 in the presence or absence of FSH on both the development of secondary follicles and the expression of mRNA for BMP-6 and FSH-R was evaluated after 6 days of culture. Furthermore, the follicular diameter and the formation of the antrum were evaluated before and after 6 days of culture. It was found that the level of mRNA for BMP-6 in primary and secondary follicles was significantly higher than those in primordial follicles. Similar levels of BMP-6 mRNA were observed in oocytes and granulosa/theca cells from small and large antral follicles. BMP-6 protein was expressed in oocytes of all categories of follicles and in granulosa cells from secondary follicles. Addition of BMP-6 into the culture medium increased the diameter of cultured secondary follicles both in presence or absence of FSH. Furthermore, addition of FSH resulted in increased levels of BMP-6 mRNA in these follicles while, together with FSH, BMP-6 administration enhanced the levels of FSH-R mRNA. In conclusion, the level of BMP-6 mRNA is increased during the transition from primordial to primary / secondary follicles, and BMP-6, like FSH, promotes growth of goat secondary follicles. Furthermore, BMP-6 increases the levels of FSH-R mRNA in cultured secondary follicles, but only in presence of FSH, which, in contrast to BMP-6, increases the level of BMP-6 mRNA herein.

P52 Developmental dynamics of ovarian cyst and persistent follicle in postpartum dairy cows

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Development of ovarian cysts and persistent follicles is one of the ovarian pathologies involved in reduced fertility of high-lactating cows. The present study characterizes the developmental dynamics and endocrinological milieu of ovarian structure formation. Multiparous cows (n=47) were monitored by transrectal ultrasonography twice a week, from 10 days postpartum to the second ovulation. Follicles with diameter ≥20mm in two consecutive ultrasonography scans were defined as potentially capable of persisting or developing into an ovarian cyst. Follicular fluids were aspirated by vaginal ultrasound transducer and stored at -20°C for hormone radioimmunoassay (RIA). All procedures were approved by the local ethics committee. Based on follicular dynamics, the examined cows were classified as having normal cyclicity (n=30; 64%) or abnormal cyclicity with development of a cyst-like structure (n=17; 36%). Based on RIA of the follicular fluid, follicles were classified as dominant (DF, n=4) characterized by high estradiol (713±140 ng/ml), high insulin (177±104 ng/ml) and low progesterone (62±22 ng/ml) concentrations with normal follicular development, or persistent (PF, n=5) with a developmental pattern like that of the DF and a slower growth rate than that of a cyst (0.82±0.1 vs. 2.3±0.28 mm/day, respectively; P<0.05), but with a large variation in steroid milieu. About 17% of the cows (n=8) developed cysts (n=16): luteal cysts with high progesterone and low estradiol (1235±439 and 116± 66 ng/ml, respectively; n=6); follicular cysts with high estradiol and low insulin (454±62 and 33±17 ng/ml, respectively; n=6), and non-steroidogenic active cysts (n=4). Following aspiration, about 38% of the cystic cows developed another cyst, while 80% of the cows with PF resumed cyclicity and underwent ovulation. It appears that early identification of non-ovular cows in the postpartum period followed by follicular aspiration of abnormal structures might reduce the proportion of such cows at the time of insemination.

P53 Activin B levels during sheep folliculogenesis, and effects on thecal androgen production

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Activin βA/βB subunits are both present in ovaries, resulting in the presence of either activin A (ßA dimer), or activin B (ßB dimer). Both activin A and B are important in developmental processes, and each appears to have a distinct role. Previously we showed that activin A suppresses androgen production from primary sheep theca cells in vitro. We now confirm a similar function for activin B, and, utilizing a newly developed ELISA (Ludlow et al) we have mapped activin B concentrations in follicular fluid collected from sheep follicles during all stages after antral development. In this study, individual follicles >1mm in diameter were collected from sheep during anestrous, in the late follicular phase just prior to the LH surge, and just after the LH surge (n=10 at each phase). Follicular fluid was collected from each follicle and activin B concentrations measured. The results showed that during anestrous, follicles of <2.5mm diameter contained approximately 750ng/ml activin B, and this concentration then decreased by half at diameters of 2.5mm or greater reaching as low as 50ng/ml by the preovulatory stage. Follicles collected just prior to ovulation contained approximately 1000ng/ml in early stages of development (<2.5mm diameter), half that at 2.5-3mm, and levels decreased to about 50ng/ml as follicles reached preovulatory size. After the LH surge, levels of activin were around 1400ng/ml in <2.5mm follicles, and again, levels decreased by about half at 2.5-3mm and again decreased to 50ng/ml in larger preovulatory follicles. In summary, the results showed that at early antral stages of development (<3mm), sheep follicles contain high levels of activin B within the follicular fluid, and the amounts of activin B decrease as the follicles continue development. These results suggest that activin B is produced by follicles at early stages of development at levels able to modulate androgen production.

P54 Progesterone production by the corpus luteum is associated with steroidogenic capacity of the dominant follicle and regulatory components of the IGF system

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Introduction: Corpora lutea (CL) formed following induced ovulation are smaller, shorter lived, produce less progesterone and are associated with lower pregnancy rates following AI than those formed following spontaneous ovulation. To test the hypothesis that these differences in CL competence are due to inherent differences in first- and final-wave dominant follicles (DF), we quantified transcript expression in somatic cells of (1) Day 6 first wave DF and spontaneously formed CL, (2) 6-day old induced CL (from Day 6 DF) and (3) final wave (pre-ovulatory) DF (Day 19).
Methods: DF and CL were dissected from ovaries of 24 heifers (8 per time point) at precise stages of synchronised oestrous cycles as specified above. Ethical approval was granted by Nottingham University. Transcript expression was quantified by GeXP (Beckman-Coulter Inc., UK) and verified by qRT-PCR. Follicular fluid oestradiol and progesterone were measured by RIA and ELISA, respectively. CL tissue was cultured to determine progesterone production.
Results and Discussion: GeXP analyses quantified transcript expression for 47 genes known to regulate DF and CL development. Progesterone and oestradiol were greater (P<0.001, P=0.002) in Day 6 than Day 19 DF, associated with increased (P<0.05) expression of *CYP19A1, CYP11A1* and *HSD3B1* in granulosa and *SCARB1* in thecal cells. Granulosa and thecal cell *IGFBP2* and *IGF2R* expression were greater (P<0.05) in Day 19 than Day 6 DF. Follicular-cell *LHCGR* expression did not differ between waves. 6-day old induced CL from Day 6 DF were smaller (P=0.02) and less (P<0.001) steroidogenic than Day 6 spontaneous CL. *LHCGR* expression was lower (P<0.05) in induced than spontaneous CL. Whereas *BMP2* and *IGFBP5* expression were higher (P<0.05) in induced than spontaneous, *GADD45B, HSD3B1, IGFBP4, KITLG, PGF2AR, STAR* and *VEGFA* expression were lower (P<0.05). These results confirm functional differences between spontaneous and induced CL associated with steroidogenic capacity of DF and regulatory components of the IGF system.

Notes

P55 Potential markers of oocyte developmental competence revealed by transcriptome analysis of cumulus cells in bovine (Bos taurus)

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Introduction: In mammals, specialised granulosa cells, named cumulus cells (CC), surround the oocyte and play essential roles in oocyte maturation and its competence to be fertilized and to support early embryo development. Assisted reproduction technologies require in vitro embryo production. However, the developmental competence of oocytes subjected to in vitro maturation (IVM) before in vitro fertilization is lower than those in vivo. The aim of this study was to compare gene expression in bovine CC after in vitro or in vivo maturation, in order to find potential markers of oocyte quality.
Methods: CC were collected from oocyte-cumulus complexes (OCCs). In vivo matured OCCs were collected from preovulatory follicles of super-ovulated cows. In vitro maturation was performed in enriched 199 medium during 24 hours on OCCs aspirated from antral follicles (3-8 mm) of cows or calves. Dye-swap hybridizations were performed on 22K bovine oligo-microarray (CRB-INRA, France) using Cy3/Cy5-labeled probes (four CC pools per condition). Real time RT-PCR was used to validate differential expression (5 independent samples per condition).
Results and Discussion: Analysis of microarray hybridizations revealed 53 up-regulated and 86 down-regulated genes in CC matured in vivo as compared to IVM (p-value<0.01 adjusted by Bonferroni method using Anapuce-R analysis). Most differential genes are known to be involved in metabolic and proteolysis processes, proliferation, stress response and apoptosis. Differential expression was confirmed by real time RT-PCR for 15 genes varied from 2.2 to 27 times between conditions. Moreover, we used calf cumulus cells after IVM as an additional model of lower developmental competence as compared to cow IVM and in vivo matured CC. Expression in CC of GSTA1 (Glutathione S-transferase A1), FSHR (FSH receptor), MMP9 (matrix metalloproteinase-9), HAS2 (Hyaluronan synthase 2) and SERPINA5 (serine protease inhibitor) varied in correlation with developmental competence of corresponded oocytes.

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P56 Expression of endogenous and exogenous hypoxic markers in sheep preovulatory antral follicles

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Previously, we have demonstrated that over 50% of sheep preovulatory antral follicles were severely hypoxic; that is, pO_2 concentrations were below 5 mmHg. About 20% of the follicles had pO_2 concentrations above normoxia. The aim of this study was to employ immunohistochemical methods to assess the presence/absence of relevant hypoxia-related antigens within the probed follicles. We hypothesised that granulosa cells of severely hypoxic (pO_2 < 10 mmHg) follicles would express the exogenous hypoxia marker pimonidazole and the endogenous hypoxic markers glucose transporter subtype 1 (GLUT1) and carbonic anhydrase 9 (CAIX). Additionally, we proposed that hypoxia inducible factor (HIF) 1α and 2α would be expressed due to their importance in mediating hypoxic responses. Glucose transporter subtype 4 (GLUT4) expression was also determined as, unlike GLUT1 and CAIX genes, it possesses no hypoxic response element (HRE). The oestrous cycles of 12 sheep were synchronised and the animals injected with pimonidazole. In the mid follicular phase after measuring the oxygen concentrations and temperature in the follicular fluid of large follicles using a tissue pO_2 sensor, the ovaries (n=24) were immediately removed, placed in 4% paraformaldehyde and then embedded in paraffin wax. Serial sections (6-7 µm) of each ovary were made. Standard immunohistochemical methods were used to detect pimonidazole, HIF1α, HIF2α, GLUT1, GLUT4 and CAIX expression in consecutive sections. The sections were viewed by light microscopy. Pimonidazole and HIF1α expression were not detected in any of the probed follicles, which was surprising as ~50% were severely hypoxic. However, HIF2α was widely expressed in the granulosa cells. There was strong positive staining for GLUT1 and CAIX in both thecal and granulosa cells and for GLUT4 in the granulosa cells of most follicles. Our next aim is to correlate the pattern of expression of these proteins to the oxygen tension in each probed follicle.

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Notes

P57 Expression and regulation of twisted gastrulation homolog 1 during preantral follicle development

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In many developmental paradigms, exposure to extracellular growth factors is modulated by the expression and secretion of specific binding proteins. In the ovary, the growth of a cohort of preantral follicles is a developmental process that also depends on the appropriate exposure to certain intra-ovarian factors. Such factors implicated are members of the bone morphogenetic protein (BMP) family. Twisted gastrulation homolog 1 (Twsg1) is a secreted protein that can enhance or antagonise BMP signalling and we found high levels of this transcript in granulosa cells of preantral follicles implying a regulatory role for Twsg1 in follicle development. The overall objective of this study was to establish how Twsg1 itself is regulated. A series of in *vitro* experiments involving preantral follicles isolated from 12-day old mice were designed to identify potential regulators: To determine the role of the oocyte, follicles were oocytectomised by micromanipulation and maintained in culture for 24hrs. In a second experiment, follicles were cultured in the presence of FSH and monitored for 72hrs. All follicles were snap frozen for quantitative RT-PCR analysis. Removal of the oocyte led to a small but significant increase in Twsg1 transcript levels in remaining granulosa cells while FSH had no effect on Twsg1 expression. Follicles were then cultured in the presence of recombinant growth differentiation factor 9 (Gdf9), a known oocyte secreted factor, yet this had no effect on Twsg1 expression alone or in combination with FSH. These results indicate that in normal preantral follicles some factor other than Gdf9 is able to partially repress Twsg1 expression, implicating the oocyte as an indirect modulator of BMP signalling in neighbouring granulosa cells.

P58 Is the presence of aquaglyceroporins -3, -7, and -9 in the bovine ovary related to follicle development?

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Introduction: Aquaglyceroporins are transmembrane proteins which selectively transport water and neutral solutes including glycerol and urea. This investigation examined the distribution of AQP -3, -7 and -9 in the bovine ovary, to assess their potential importance to the developing follicle and oocyte.
Method: Ovaries and positive control tissues were placed in neutral buffered formalin within five minutes of slaughter. Tissues were processed, paraffin-embedded and sectioned at 5µm. The DakoCytomation EnVision+ Dual Link–HRP (DAB+) kit was employed for immunohistochemical studies, using a panel of rabbit polyclonal antibodies raised against immunogenic peptides from rat aquaporins (C- or N-terminal sequences). Follicles were classified for stage and Type using the Braw-Tal & Yossefi system.
Results and Discussion: AQP-3, -7 and -9 were abundant in oocytes at all follicle stages and in peripheral nerves, vascular smooth muscle and some endothelia. AQP-3 was present in the granulosa of Type 1 – Type 4 follicles and in columnar mural granulosa of large antral follicles, yet absent from the granulosa of type 5 follicles and large antral follicles with rounded mural cells. AQP-3 was present in theca from early Type 5 through to large antral follicles. AQP-7 was present in granulosa from Type 1 – Type 3 follicles and in mural granulosa of large luteal phase (LP) follicles but absent from mural granulosa of large follicular phase (FP) follicles. It occurred in theca of Type 5 and large FP follicles but not in large LP follicles. AQP-9 was found in granulosa of Type 1 – Type 3 and large antral follicles, in theca of large FP follicles but not large LP follicles. These results show that aquaglyceroporins are abundant in follicles and oocytes at all developmental stages. Specific AQPs appear in granulosa and theca cells in a stage-related manner, related to granulosal layering, antrum formation and the absence/presence of atresia.

Notes

P59 Polyunsaturated fatty acids modify prostaglandin and steroid output from bovine ovarian cells in vitro

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Although polyunsaturated fatty acids (PUFAs) form ~7% of the total energy in a typical western diet, this comprises a fraction of their overall physiological role. PUFAs are classified as n-3, n-6 or n-9 according to the position of the first carbon-carbon double bond. They act as substrates to the cyclooxygenase enzymes (COX-1/2) for synthesis of prostaglandins (e.g. prostaglandins E2:PGE2 and F2α PGF2α), important regulators of ovarian steroidogenesis. PUFAs also modulate steroidogenic acute regulatory protein (StAR) which affects steroidogenesis. This study investigated the ability of n-6 PUFAs to modify ovarian steroid and prostaglandin synthesis. Bovine granulosa cells from medium sized follicles were isolated and cultured at a density of 3x106/ml for 96h in McCoy's 5A medium with supplements. At 48h, cells were treated with 100μM of the n-6 PUFAs linoleic acid (LA) or arachidonic acid (AA) with or without 100μM indomethacin (IMC; COX-1/2 inhibitor) or 10μM SC560 (COX-1 inhibitor). PGE2 and progesterone (P4) in spent culture media was determined by radioimmunoassay while COX-1/2 and StAR mRNA in cell extracts were analysed by qPCR. Statistical significance was determined by a one-way ANOVA ($p<0.05$). LA and AA stimulated P4 secretion and this effect was abolished by SC560. AA (but not LA) stimulated expression of StAR, COX-2, and secretion of PGE2 ($p<0.05$). As expected, SC560 and IMC inhibited basal PG levels but, surprisingly, IMC increased basal P4 secretion ($P<0.05$) whereas SC560 had no effect on P4. Moreover, LA and IMC together enhanced P4 whilst AA and IMC together did NOT further increase P4. These data suggest that n-6 PUFAs affect P4 synthesis through both direct and indirect mechanisms: LA stimulates steroidogenesis independently of StAR and PGE2 whereas AA stimulates both PGE2 and StAR in inducing steroidogenesis. However, we have yet to establish whether the PG-independent effects are mediated directly through actions on StAR expression.

P60 The presence and distribution of aquaporin -1, -4 and -5 in the bovine ovary is cell type- and follicle stage-dependent

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Introduction: Water and solute transport through tissues is mediated by aquaporins (AQPs), a family of transmembrane channel proteins. AQP-1, -2, -4, -5, -6 and -8 are primarily water permeable and possible routes for fluid transfer during ovarian follicle development. This study localised AQP-1, -2, -4 and -5 in the bovine ovary with the aim of understanding their potential role in folliculogenesis and oocyte maturation.
Method: Ovaries and positive control tissues were placed in neutral buffered formalin within five minutes of slaughter. Tissues were processed, paraffin-embedded and sectioned at 5μm. The DakoCytomation EnVision+ Dual Link–HRP (DAB+) kit was employed for immunohistochemical studies, using a panel of rabbit polyclonal antibodies raised against immunogenic peptides from rat aquaporins (C- or N-terminal sequences). Follicles were classified for stage and Type using the Braw-Tal & Yossefi system
Results and Discussion: AQP-1 occurred in microvessel endothelia, peripheral nerves and erythrocytes but not follicular tissue. AQP-2 was undetectable (but present in kidney control). AQP-4 and -5 occurred in oocytes at all follicle stages and in peripheral nerve, endothelium and vascular smooth muscle. AQP-4 was found throughout the granulosa in Type 1 to Type 4 follicles and in granulosa of large antral follicles but was absent from Type 5 follicles. It occurred in the theca of large antral follicles during the follicular but not luteal phase. AQP-5 also occurred throughout the granulosa of Type 1 to Type 4 follicles but was restricted to mural cells of Type 5 and large antral follicles. It was present in theca layers at all stages except large luteal phase follicles. These results suggest that specific AQPs are involved in oocyte function and the supply of fluid to follicles and other structures. AQPs -4 and -5 occur in follicular cells in a stage-related manner, suggesting active fluid regulation during follicle development.

Notes

P61 Oocyte-specific deletion of mucin O-glycans modifies the form and function of the follicle basal lamina

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Introduction: Mice with oocyte-specific deletion of O-glycans (*T-syn* mutant) have increased fertility and numerous multiple-oocyte follicles (MOFs). MOFs appear to form by adjacent follicles joining, likely involving basal lamina (BL) remodelling. It is also unknown if MOFs can develop to the preovulatory stage. We aimed to establish if BL structure and/or function are modified and if MOFs ovulate in *T-syn* mutants.
Methods: BL structure was investigated in ovary sections from control and mutants using Periodic Acids Schiff which stains the BL pink. The BL was classified as 'defined' if it was continuous and clear, or 'undefined' if unclear. BL function was assessed using immunohistochemistry to detect intrafollicular IgG; an endogenous molecule partially excluded from follicles. To assess preovulatory MOF development, control and mutant females were superovulated and ovaries collected prior to ovulation. Ovaries were sectioned (5μm) and every 5th section analysed identifying all preovulatory follicles and counting oocytes. Corpora lutea (CL) number and morphology were also analysed.
Results and Discussion: The proportion of mutant follicles with an undefined BL, which appears to fuse with the stromal extracellular matrix, increases with the stage of development compared to controls which remains constant. An increase in BL layers correlates with reduced fertility; in mutants the quantity of BL layers appear to decrease, consistent with the increase in fertility. IgG in mutant follicles is decreased at all stages except antral compared to controls. Therefore mutant follicles are less permeable to IgG than controls; alternatively more IgG is degraded within mutant follicles. The mutant did not have increased numbers of preovulatory MOFs compared with controls, thus demonstrating that most MOFs cannot develop to ovulatory follicles. More mutant CL had a lumen compared to controls which could be due to changes in follicular proteoglycans. In summary, oocyte O-glycans alter follicle development and function.

P62 Establishing a model of ovarian cyst development in the bovine ovary

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Introduction: Ovarian cyst formation is a major cause of infertility in cattle, but little is known of the aetiology of this ovarian pathology. Previous work has shown that formation of ovarian cysts is commonly observed after prolonged down-regulation of the hypo-pituitary axis with GnRH-agonist. The aim of this study was to examine the endocrine and physiological changes associated with ovarian cyst development in this experimental model system.
Method: The study was carried out in 12 heifers previously down regulated with GnRH agonist (buserelin) for 7 weeks followed by one cycle of ovarian stimulation designed to induce ovulation. Ovarian and endocrine function was then monitored by daily ultrasound and 8 hourly blood sampling for 120 h following administration of a luteolytic dose of the prostaglandin F2a analogue, cloprostenol. Animals were then sacrificed and ovaries collected and follicles dissected. Peripheral hormone concentrations were measured by immunoassay and expression of aromatase by immunohistochemistry.
Results and Discussion: Six animals exhibited an LH surge within 104 hours of luteal regression while 6 animals did not, though no animals had evidence of fresh ovulation points at slaughter 16 h later. FSH concentrations showed divergence comparable with LH surges. While animals exhibited variable follicle development all heifers had at least 1 follicle > 8 mm and 5/12 had at least 1 follicle > 20 mm. Follicle appearance ranged from healthy, well vascularised to heavily luteinised with 63% of follicles showing some degree of luteinisation. Aromatase activity was only detected in 22% of follicles. In conclusion, these results confirm that ovarian cyst-like structures develop at a high frequency following prolonged GnRH-agonist suppression and that their formation is associated with both endocrine imbalance and abnormal ovarian function. This experimental model system may, therefore, be useful in the study of this ovarian pathology.

Notes

P63 Gap junction communication is essential for maintenance of intracellular cAMP in rat COCs

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Within the developing ovarian follicle, oocyte maturation is reliant upon bi-directional communication between the oocyte and adjacent cumulus cells[1,2]. In the present study, cumulus-oocyte complexes (COC) were isolated from small antral follicles of rats at day 21-25 and the degree of intercellular gap junctional connections with respect to time in culture or after administration of various treatments was assessed by measuring the extent of transfer of the fluorescence indicator, Calcein from cumulus cells to the oocyte. To determine the effect of time, groups of 10 COCs were pulsed with Calcein-AM (1μM) for 10 min before culture in phenol red-free M199 medium supplemented with the PDE inhibitor, milrinone (100 μM) for 0, 2, 4, 8, 12 and 24 hours. Thereafter, the oocytes were denuded (DO) of their cumulus cells, and the fluorescence intensities of the DOs measured and normalised against those at 0 hours. The optimal time of transfer of Calcein from the cumulus cells to the oocyte, was 4hours ($P<0.01$), after which time the fluorescence intensity declined ($P<0.05$). To determine the effects of db-cAMP (1mM), rhFSH (250IU/mL), Forskolin (10μM), IGF-1 (10ng/mL), oestradiol (1μg/mL), testosterone (1μg/mL), androstenedione (1μg/mL) and progesterone (1μg/mL) on the rate of Calcein dye transfer via gap junctions over 4hours, groups of 10 rat COCs were pulsed with Calcein (1uM) as described above before culture in phenol red-free M199 media supplemented with the various treatments or the vehicle alone for 0 and 4hours. Immediately thereafter, the fluorescence intensities of the DOs were measured and normalised against the 0 time values. Calcein dye transfer increased after treatment of COCs with db-cAMP ($P<0.05$), rhFSH ($P<0.05$) and Forskolin ($P<0.05$), whilst treatment with IGF-1, oestradiol, testosterone, androstenedione and progesterone had no effect. We conclude that all treatments which increase the intracellular concentrations of cAMP promote gap junction communication in rat COCs.

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P64 Kisspeptin-10 inhibits angiogenesis: a role in the placenta

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Background: Kisspeptin is a neuropeptide known to play a role in the regulation of gonadotrophin secretion. Kisspeptin, transcribed from the KISS1 gene, is a potent inhibitor of tumour metastasis and plays a role in placentation; both processes involving angiogenesis. In addition, kisspeptin and its receptor, KISS-1R, have been identified in human blood vessels (aorta, coronary artery, umbilical vein), where they mediate vasoconstriction. We, therefore, hypothesised that kisspeptin plays a role in regulating angiogenesis in the reproductive system.
Methods: KISS1R expression in the human umbilical vein endothelial cells (HUVEC) and in the placenta were determined using immunochemistry. The effects of kisspeptin-10 (KP-10, a shorter biologically-active cleavage peptide) at 100 pM - 1 μM concentrations were tested on endothelial cell viability, apoptosis, tube formation, proliferation and migration in vitro using HUVEC. The influence of KP-10 on new vessel sprouting was tested in an ex vivo model of angiogenesis using human placental arteries embedded in Matrigel.
Results and Discussion: Immunochemistry identified KISS-1R in HUVEC and placental blood vessels. KP-10 had no effect on HUVEC viability or apoptosis. In contrast, proliferation and migration of HUVEC were inhibited by the highest concentration (1μM) of KP-10. KP-10 also inhibited tube-like structure formation by HUVEC in a concentration-dependent manner, after 8 and 24 hours, in culture. Similarly, KP-10 (1μM) inhibited new vessel sprouting from the placental vessel In conclusion, these in vitro and ex vivo results show that KP-10 has anti-angiogenic effects in the reproductive system. Thus KP-10 may play a role in regulation of angiogenesis in the placenta, where this process is tightly controlled. This may have implications for pathologies in which angiogenesis is dysregulated, such as pre-eclampsia and intra-uterine growth retardation.

Notes

P65 Effect of breed and nutrient supply during pregnancy on testis development in sheep

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Introduction: We have shown that undernutrition during early and mid-pregnancy does not affect testis size or testosterone concentrations in ram lambs (Rooke et al., 2008). This study assessed whether such undernutrition altered the cellular composition of the testis and if there was a breed difference in response.
Methods: Suffolk (S) or Scottish Blackface (SB) ewes were mated to sires of the same breed and fed either an adequate diet (C), or a restricted diet (R) supplying 1.0 or 0.7 of maintenance energy requirements respectively from days 1 to 90 of gestation. All ewes received maintenance diets during the remainder of pregnancy and lactation. Testes from 32 37-week old rams (n=8 per breed/treatment combination) were collected at slaughter, preserved in Bouin's solution, sectioned and stained with haematoxylin and eosin. Numbers of Sertoli cells and spermatagonia were counted and seminiferous tubule diameter and area measured. Animal studies were conducted in accordance with the Animals (Scientific Procedures) Act, 1986.
Results and Discussion: Both ewe breed and nutritional treatment affected the number of spermatagonia per seminiferous tubule (SBC 29.02±0.97; SC: 30.44±0.94; SBR: 22.09±0.35, SR: 25.45±1.51, breed: $P<0.05$; nutrition: $P<0.001$). Male lambs born to R ewes had fewer Sertoli cells when expressed per seminiferous tubule (21.34±0.55 versus 13.84±0.61; $P<0.001$) and per area of testis tissue (0.97±0.007/nm2 versus 0.60±0.003/nm2; $P<0.001$) than lambs carried by C ewes. These results indicate that undernutrition during pregnancy reduces the number of spermatagonia and Sertoli cells in the absence of effects on testis size or testosterone concentrations. As maximum Sertoli cell numbers are established by day 80 of life and their number determines maximal sperm production, prenatal undernutrition may affect adult male reproductive capacity. Reference: Rooke, J.A., McIlvaney, K., Dwyer, C.M. & Ashworth, C.J. (2008) Reproduction in Domestic Animals 43: P169. (Funded by the Scottish Government and Edinburgh University).

P66 Pre-natal stress and post-natal pain affect testis development in young pigs

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Introduction: There is increasing evidence that the developing reproductive axis is sensitive to stressors encountered during early life. This study examined the effects of fetal exposure to maternal social stress and/or transient post-natal pain on the developing pig testis.
Methods: 8 pregnant sows were stressed by mixing with unfamiliar older sows for 2 1-week periods during mid-pregnancy (M) while 8 control pregnant sows (C) were not mixed. On day 3 after birth, half the piglets from each litter had their tails amputated (D), while the other piglets were left intact (I). Testicular tissue and plasma was obtained from 1 D and 1 I male piglet from each litter at slaughter on 40.44±0.27 days of age. Plasma concentrations of testosterone were determined by radioimmunoassay. Testes were preserved in Bouin's solution, sectioned and stained with haematoxylin to count numbers of seminiferous tubules and Leydig, Sertoli and germ cells. Immunohistochemistry of Ki67 was conducted to estimate numbers of proliferating cells. Data were analysed in a 2x2 analysis of variance, fitting age at slaughter as a covariate. Animal studies were conducted in accordance with the Animals (Scientific Procedures) Act, 1986.
Results and Discussion: Pigs born to M mothers had lower testosterone concentrations (1.88±0.34 versus 2.97±0.40 nmol/L; $P=0.023$) and tended to have more non-proliferative cells in their testes (31.16±1.34 versus 24.85±2.10%, $P=0.057$) compared to piglets born to C mothers. When compared with intact littermates, tail amputation reduced testis size (0.101±0.004 versus 0.091±0.005% of body weight; $P=0.01$) and the percentage of proliferating Leydig cells (30.08±3.25 versus 22.95±3.35%; $P=0.03$). There were no effects on other cell types and no interactions between pre- and post-natal treatment. These data suggest that the steroidogenic function of the pig testis is sensitive to early life experience.

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Notes

P67 Upregulation of autophagy genes (ATGs) in developing chorioallantoic tissues (CA) of embryos produced by Assisted Reproductive Technologies (ART)

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The use of ART has been associated with an increased risk of adverse pregnancy outcomes. Several reports demonstrated placental defects in ART pregnancies, however the origin of these abnormalities remains unknown. We observed that already early postimplantational (day 20-24 of gestation) sheep embryos obtained by ART are smaller in diameter than controls obtained by natural mating. ART embryos were produced entirely in vitro using oocytes maturation, fertilization and embryo culture protocols routinely used in our laboratory. Day 6 embryos were transferred to synchronized recipient sheep for the establishment of pregnancy. Embryos and CA were collected for molecular analysis at days 20-24 of pregnancy. In order to check if the retarded growth of ART embryo can be attributed to deregulated metabolism in its placenta we measured mitochondrial activity in ART CA. Analysis confirmed significantly lower (P=0.01) level of functional mitochondria in ART CA. This low rate of mitochondrial activity can be associated with deregulation of cellular death pathways such as apoptosis and autophagy, both playing the main role in growth regulation and tissue remodelling. In order to check if any of these two cellular pathways are deregulated in ART CA we analyzed by Real-time PCR, the expression levels of genes regulating autophagy and apoptosis. All analyzed autophagy genes (ATG-5, BECLIN-1, ATG-9) were upregulated in ART CA ($p<0.05$, Mann-Whitney t-test), however no difference in expression of other related to autophagy genes (LC-3, AMBRA1, UVRAG, ULK1, ULK2) was noted. Furthermore, one of 4 proapoptotic genes, BAX, acting in outer mitochondrial membrane was up regulated ($p<0.05$, Mann-Whitney t-test) in ART CA. We show that autophagy genes are upregulated in early CA. To our knowledge there no other reports about autophagy regulation in early placentae. Our data suggests that the use of ART causes deregulated autophagy in CA, which can lead to aberrant placental development.

P68 Low shear stress in first trimester spiral arteries may facilitate trophoblast induced endothelial cell apoptosis

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Objective: In the first trimester of pregnancy trophoblast migrate along uterine spiral arteries (SA) and replace endothelial cells (EC) lining these vessels by mechanisms involving EC apoptosis. Inadequate SA remodelling has been associated with pre-eclampsia and IUGR. Until 10-12wks of gestation trophoblast plug SA, preventing maternal blood flow into the intervillous space, and resulting in high-resistance flow in these vessels. This work aimed to determine the effect of shear stress on trophoblast-induced EC apoptosis.
Methods: Cells were cultured using the BioFlux200 system. SGHPL-4, Jar (trophoblast cell lines) and HUVECs were cultured with/without TNFα/Actinomycin-D for 12-30hrs under shear stresses from 0.02-7dyne/cm. Apoptosis was quantified by timelapse microscopy.
Results: 1) Shear stresses from 0.02- 3dyne/cm did not affect the rate of apoptosis of SGHPL-4, Jar or HUVECs (0-10% death). 2) TNFα/Actinomycin-D treatment significantly increased apoptotic cell death in all cell types ($p<0.01$). However, trophoblast cultured in 3dyne/cm underwent significantly less apoptosis than those in 0.5dyne/cm cultures ($p<0.05$). This protective effect was seen to a small extent in HUVECs cultured at 3dyne/cm, but was not significant. 3) Jars cultured on HUVEC monolayers at 0.5 or 3dyne/cm significantly induced apoptosis in directly adjacent HUVECs, in comparison to HUVECs >2 cells away or HUVEC only controls ($p<0.05$), with more apoptosis induced in 0.5 than 3dyne/cm cultures ($p<0.05$). However, Jars failed to significantly induce HUVEC apoptosis in 5 or 7dyne/cm cultures.
Discussion: Trophoblast demonstrate a survival advantage over HUVEC at low levels of shear stress (3dyne/cm). Jars are able to induce HUVEC apoptosis in this model in a similar manner to trophoblast *in vivo*, and this process is inhibited by increasing shear stress. Therefore, low shear stresses in plugged SA in the first trimester may aid trophoblast-induced EC apoptosis involved in SA remodelling.

Notes

P69 Expression of short chain fatty acid receptor GPR43 in utero-placental tissues during labour

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Introduction: The G-protein coupled receptor GPR43 (FFA2) is one of a family of receptors, which are activated by short chain fatty acids. GPR43 is highly expressed in immune cells and has been suggested to be involved in inflammatory responses. Normal labour is associated with up-regulation of inflammatory genes and influx of immune cells into the myometrium and cervix. In the present study we have investigated the temporal expression and site of localisation of GPR43 in utero-placental tissue from pregnant women at term before the onset of labour or during labour.
Methods: Myometrium, placenta, chorion and amnion tissues were obtained from women not in labour and in labour at term (>37 weeks gestation), in accordance with approval from the Lothian Research Ethics Committee and with informed written consent from all study patients. GPR43 mRNA and protein expression were determined in all tissues by quantitative RT-PCR and immunohistochemistry.
Results and Discussion: Expression of GPR43 mRNA was significantly higher in the myometrium, chorion and amnion collected from women in labour compared to tissue collected from women at term but not in labour ($p<0.05$). GPR43 mRNA expression in placental tissue did not differ between the two groups. GPR43 protein was localised by immunohistochemistry to leucocytes and the vascular endothelium in the myometrium, chorion and amnion. Expression was also localised in the epithelium of the amnion and in the syncytiotrophoblast layer and foetal blood vessels in the placenta. These data demonstrate an elevated expression of the pro-inflammatory GPR43 in tissues of the utero-placental unit with the onset of labour. The functional significance of this elevation and the role of GPR43 in labour remains to be elucidated.

P70 Dilution of stallion spermatozoa for transport prior to sex-sorting

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Introduction: Stallion spermatozoa are stained with fluorescent dye at a concentration of 111×10^6/mL for flow cytometric sex-sorting. Previous studies have demonstrated that the preferred concentration for shipping stallion spermatozoa is between 25 and 100×10^6/mL. Consequently, this necessitates a centrifugation step to concentrate spermatozoa prior to sex-sorting. The aim of this experiment was to determine the optimal protocol for transporting stallion spermatozoa for a final concentration of 111×10^6/mL.
Materials: Dilutions were performed using Kenney's Modified Tyrodes and all centrifugations were at $350 \times g$. Split ejaculates (N=9) were either diluted to 25×10^6/mL without washing to remove seminal plasma (+SP25), washed via centrifugation and diluted to 25×10^6/mL (-SP25) or washed and diluted to 111×10^6/mL (-SP111). After storage at 15ºC for 18 h (all treatments), +SP25 and –SP25 were centrifuged and resuspended to 111×10^6/mL. Spermatozoa were then incubated at 34ºC for 1h to simulate staining incubation prior to sex-sorting. Motility (CASA), viability and acrosome integrity (fluorescence microscopy) were assessed following storage (0h) and incubation (1h).
Results and Discussion: There were no significant differences in the viability or acrosome integrity of spermatozoa for any treatment. Total motility for –SP111 was lower than +SP25 and –SP25 at 0h (58.6 vs. 75.0 and 79.3%, $p<0.05$), and progressive motility was lower for –SP111 than +SP25 and –SP25 at 0h (4.0 vs. 8.0 and 11.7%, $p<0.05$) and 1h (8.8 vs. 12.8 and 14.2%, $p<0.05$). An additional centrifugation step to concentrate spermatozoa before incubation induced less damage than storage at the higher concentration required for sex-sorting. The results suggest the negative effects of seminal plasma were ameliorated by dilution of spermatozoa to 25×10^6/mL. The recommended protocol for transporting stallion spermatozoa at 15ºC for sex-sorting is to dilute the ejaculate to 25×10^6 sperm/mL without washing.

Notes

P71 Embryonic Stem Cells: Modelling Effects of Early Embryo Environment on Developmental Potential

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Introduction: Poor maternal diet at critical stages of development, particularly the preimplantation period, induces physiological, metabolic and morphological alterations in offspring which can contribute to adult onset disease in later life. Changes likely occur within the distinct stem cell populations of the embryo which would remain conserved throughout development. Here, embryonic stem cells (ESCs) derived from early embryos are used to explore mechanisms involved in adaptive responses to maternal diet. Initially we compare the derivation of ESCs and their proliferative capability and metabolic activity.
Methods: ESC lines were derived from blastocysts of C57BL/6 mice assigned to either a low protein diet LPD (9% casein, n=13) or a normal protein diet NPD (18% casein, n=13) exclusively throughout the preimplantation period of development. Cells were routinely cultured in Knockout-DMEM containing 15% Knockout-Serum replacement plus LIF (1000 U/ml). Preliminary studies compared the relative derivation, proliferation rates and metabolic activity of ESCs across treatments. Cell proliferation was assessed by conventional haemocytometer counts at 24hr intervals over a total of 96hrs. Metabolic activity was determined over 96hrs using assays based on the transformation and colorimetric quantification of MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide]. In other assays, ESC gender, karyotype, gene and protein expression patterns, differentiation capacity and epigenetic status are under examination.
Results and Discussion: The success rate of ESC isolation was significantly reduced by maternal LPD (17.4% vs. 41.9% in NPD, P0.02). This distinction may indicate an alteration in the number or developmental potential of pluripotent cells present. However, dietary treatment did not affect cell proliferation or metabolism, suggesting equivalence in the pre-differentiation status of these cell lines. Furthermore, previous data has shown gender specific responses of embryos to diet. Separating this data by sex may expose a dietary response.

P72 Prokineticins may be regulators of human fetal testis development

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Introduction: The Prokineticins (PROK1 and PROK2) are regulators of key cellular processes including proliferation, differentiation, migration, immune response and tissue-specific angiogenesis. The PROKs signal interchangeably via two G-coupled protein receptors: Prokineticin receptor 1 and 2 (PROKR1 and PROKR2). Both ligands are highly expressed in the adult human and rodent testis. It has been postulated that PROKs may play a role in development of the testis vasculature and exert anti-apoptotic effects on fetal germ cells. We have therefore investigated the expression and function of PROK signalling in the human fetal testis to determine roles during development.
Methods: Testes were dissected from human fetuses (8-20 weeks gestation) obtained with informed consent following termination. qRT-PCR and immunofluorescence was performed across gestational ranges using standard methods.
Results and Discussion: The *PROK* ligands and receptors were detected by qRT-PCR at all gestations. Expression of *PROK1* and *2* is developmentally-regulated; expression was low during first trimester, but increased significantly across gestation (p=0.03 and 0.02 respectively). *PROKR1* and *PROKR2* also showed significant increases in expression during the late second trimester (p=0.009 and 0.004 respectively). Immunohistochemistry was performed to determine specific sights of PROK action. PROK ligands were expressed in both intratubular and interstitial compartments of the human fetal testis. Expression of the PROK receptors was more limited, with both co-localising strongly with Smooth Muscle Actin (SMA; a marker of fetal blood vessels), supporting the hypothesis that the PROKs drive testicular vascularisation. PROKR1 was also strongly expressed within semineferous tubules at later gestations, supporting a potential role in germ cell survival. Co-localisation with markers of specific cell types will establish whether the effects on germ cells are direct, or mediated through Sertoli cells. These data suggest a possible role for PROK signalling in human fetal testis development.

Notes

P73 The expression of aquaporin 7 in the ovine fetal epididymis is perturbed by in-utero exposure to a cocktail of chemicals contained in sewage sludge fertiliser

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Introduction: Exposure to environmental endocrine disrupting chemicals (EDCs) during pregnancy in animals and humans is associated with reduced sperm counts and increased incidences of testicular cancer and reproductive tract abnormalities. Although male fetuses from pregnant ewes exposed to EDCs contained in sewage sludge fertiliser (a “real life” exposure model) exhibit fewer testicular Sertoli and Leydig cells, the epididymis has not been investigated. The movement of fluids and small solutes in the epididymis is critical for adult sperm maturation and this process is regulated, in part, by the aquaporins. The current study examined the effects of sewage sludge exposure on the expression of aquaporins in the late gestation fetal epididymis.
Methods: Pregnant ewes were exposed to sewage sludge fertiliser or control pastures from 0-140 days of gestation. Epididymides were collected post mortem at day 140 of gestation (term = day 145), Bouins-fixed and subjected to immunohistochemistry for Aquaporins 1,4 and 7. Staining intensity was visualy assessed (blinded) by 2 observers on an arbitrary 4 point scale. [Animal studies approved by local ethical committee and covered by UK Animals Scientific Procedures (1986)].
Results and Discussion: AQP1 immunoreactivity was detected in the apical and lateral plasma membranes of non-ciliated cells in efferent ducts; smooth muscle cells surrounding epididymal ducts and microvascular endothelia throughout the epididymis. AQP4 was immunolocalised to the efferent ducts and throughout the epididymis and AQP7 was detected primarily in the efferent ducts. Sewage sludge exposure significantly reduced AQP7 immunostaining (P=0.012) but had no effect on AQP1 or AQP4 in terms of localisation or immunostaining intensity. These results suggest that exposure to environmental chemicals may affect the capacity of efferent ducts to transport water and small solutes thus influencing fluid resorption, protein synthesis and secretion. Ultimately, this may impact on sperm viability in the adult.

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P74 The detection of apoptotic testicular cells on archived paraffin-embedded histological sections

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Introduction: Studies conducted in our laboratory have shown that mice treated in *utero* with ethyl nitroso urea at the stage of primordial germ cell migration show increased levels of genetic damage and impaired spermatological parameters as adults. Interestingly, although the levels of genetic damage reduce as the mice become more mature, the sperm defects do not improve. In order to determine the role of apoptosis in mediating this effect, we compared the amount of apoptosis occurring in ENU-treated animals of different ages. This involved examining testes that had been paraffin-embedded about 5 years previously.
Materials and Methods: It was discovered that in following the standard protocol we use for detecting apoptosis in testicular cells, a residue remained on the slides after de-paraffinisation. The detection of apoptosis did not work on these sections so a variety of methods was employed to remove the residue. These included using extended histoclear washes, 2-butanol, hexane, microwave-treatment and heating but none was effective. However, increasing the number of units of deoxynucleotidyl transferase enzyme in the reaction mixture did restore the efficacy of the method so all samples were tested with the modified protocol.
Results and Discussion: Apoptotic cell numbers were normalized against Sertoli cell numbers and compared per tubule per group. An increase in apoptosis was found in the highest dose ENU-treated group compared with the controls but no others. There was no significant difference in levels of apoptosis among samples from the later time point at any dose. This supports the hypothesis that apoptosis of damaged stem cells is responsible for the lowering of genetic damage in the germ-line seen at the same time-point.

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Notes

P75 The effects of a high fat high cholesterol diet on markers of uterine contractility during parturition in the rat

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Introduction: Obesity is a major public health concern in the UK and its prevalence in women of reproductive age is increasing. Approximately 13% of women aged below 30 and 22% of 31 to 40 year olds were obese in 2007. Obesity causes complications during pregnancy and increases the risk of emergency caesarean section due to prolonged labour and poor uterine activity. This studies aim was to investigate whether diet induced obesity decreases markers of uterine contractility during parturition in the rat.
Methods: Female Wistar rats were fed a control (CON n=10) or a high-fat high-cholesterol (HFHC n=10) diet for 6 weeks. Animals were then mated, and once pregnancy confirmed, maintained on their CON or HFHC diets throughout gestation. On gestational day 19, rats were monitored continuously and euthanized at onset of parturition. Body and fat depot weights were recorded, and myometrial tissue and plasma samples snap frozen and stored at -80°C for analysis of cholesterol (CHOL), triglycerides (TAG), prostaglandin F2α (PGF2α) and expression of the contractile associated proteins connexin-43 (C43), cyclo-oxygenase 2 (COX-2) and caveolin 1 (CAV-1).
Results and Discussion: HFHC fed rats gained significantly greater weight than CON (321 ± 3.4g versus 298.9 ± 9.9g P<0.031) and exhibited significant increases in peri-renal fat (P<0.01). The HFHC diet increased plasma levels of CHOL and TAG but decreased PGF2α when compared to CON (P<0.01, P<0.01 and P<0.05 respectively). In contrast, myometrial levels of CHOL and TAG were not dissimilar. However, myometrial tissue from HFHC fed rats showed a significant increase in COX-2 (P<0.037), but decreases in C43 and CAV-1 (P=0.059). In conclusion a HFHC diet significantly increases bodyweight and alters lipid profiles that correlate with decreases in key markers of uterine contractility during parturition. Further work is required to ascertain whether these changes have adverse effects on uterine activity.

P76 Novel functions for prostaglandin F receptor in endometrial remodelling

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Introduction: Prostaglandin (PG) F2alpha mediates its effect in target cells via a GPCR termed PTGFR. We have previously demonstrated peak expression of PTGFR in the human proliferative endometrium and elevated expression in endometrial cancer. PTGFR expression localises predominantly to glandular epithelium as well as the stromal cells and vasculature. This study was designed to elucidate the function of PTGFR in the human endometrium.
Methods: A human endometrial epithelial cell line (Ishikawa) was stably transfected to express either: full length PTGFR cDNA (PTGFR cells), lentiviral constructs containing miRNA sequences to ablate PTGFR (miRNA cells) or control lentiviral cDNA construct (WT cells). AB1700 v.2 Applied Biosystems Human Genome Survey microarrays were used to identify global changes in transcription levels in PTGFR cells treated with 100 nM PGF2alpha. WT, PTGFR and miRNA cells were xenografted by subcutaneous injection into CD1-Foxn1nu immunocompromised mice. Tumour architecture was analysed after 6 weeks of growth by immunohistochemistry with markers for myofibroblasts (alpha SMA), endothelial cells (CD31) and Masson's Trichome stain to quantify collagen deposition.
Results and Discussion: Microarray analysis of PTGFR cells treated with PGF2alpha identified over 700 differentially regulated genes. Gene ontology database analysis revealed three main themes, namely cell motility/chemotaxis, immune cell and blood vessel function. PTGFR expression was significantly different between the tumours that had arisen from the 3-cell types (PTGFR>WT>miRNA; P<0.05). Stereological analysis of xenograft tumours demonstrated that with increased PTGFR expression blood vessel size decreased (P<0.01) whereas myofibroblast cell number and collagen deposition increased (P<0.01). These results demonstrate that PTGFR has a significant impact on myofibroblast recruitment and/or proliferation, collagen deposition and regulation of blood vessel architecture. These processes may have functional implication in endometrial remodeling following menstruation.

Notes

P77 Novel roles for prokineticin 1 in human parturition and its premature onset

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Introduction: It is well recognised that the onset of labour is associated with increased expression of inflammatory mediators and influx of leucocytes into the myometrium and cervix. Preterm labour is often associated with an infection and premature induction of inflammatory pathways. Prokineticins (PROK1 and PROK2) are multifunctional secreted proteins demonstrated to regulate inflammatory pathways and smooth muscle contractions via the G-protein coupled receptors, PROKR1 and PROKR2. In this study, we aimed to investigate the expression of PROK1 and PROKR1 in the human myometrium in labour and their potential regulation by a mimetic of infection (lipopolysaccharide; LPS).
Methods: Myometrium tissue was collected from pregnant women not in labour and in labour at term (>37 weeks gestation), in accordance with approval from the Local Research Ethics Committee and informed written consent from all study patients. mRNA and protein expression of genes were assessed by RT-PCR and immunohistochemistry. Myometrial biopsies from women at term but not in labour were treated with vehicle, 10-1000 ng/ml LPS or 40 nM PROK1.
Results and Discussion: PROK1 mRNA expression was elevated in myometrial tissue in labour (p<0.05) whereas expression of the PROKR1 did not change. PROK1 and PROKR1 were localised by immunohistochemistry to the smooth muscle bundles, vascular endothelium and leucocytes. Treatment of myometrial tissue with LPS increased mRNA expression of PROK1 and PROKR1 (p<0.05). Moreover, treatment of myometrial explants with 40 nM PROK1 increased mRNA expression of key inflammatory mediators such interleukin (IL)-6, IL-8 and cyclo-oxygenase (COX)-2 (p<0.05). These data highlight that PROK1, via upregulation of pro-inflammatory pathways may have an important role in the initiation of parturition. The regulation of PROK1 and PROKR1 by a mimetic of infection such as LPS indicates PROK1-PROKR1 may play an important role in infection-induced premature onset of parturition.

P78 Localisation and expression of Secreted Phosphoprotein 1 in porcine uterine and placental tissues

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Introduction: We have previously identified a QTL on chromosome 8 associated with litter size in the pig (Hernandez, et al., 2009). The gene for Secreted Phosphoprotein 1 (SPP1 or osteopontin) is located under this QTL peak. SPP1 has a key role in conceptus implantation and maintenance of pregnancy. This study was conducted to test the hypothesis that differences in fetal growth may be associated with the effectiveness of conceptus attachment, as measured by SSP1 expression.
Methods: Placental, endometrial and whole uterine tissues supplying the smallest and an average-sized fetus within the same litter were collected from 9 Large White-Landrace gilts or sows between days 40 to 45 of pregnancy (term = 114 days). RNA was isolated from these tissues and qPCR performed to quantify SPP1 expression. The assessment of spatial expression of SPP1 protein and mRNA in these tissues was performed by IHC and ISH respectively.
Results and Discussion: SPP1 protein expression was localized in the endometrial glandular epithelium, with lower expression in the luminal epithelium. SPP1 mRNA was expressed in the same areas as the protein in the endometrium but with a different pattern. There was a significant difference between tissues for SPP1 mRNA expression (placenta: 21.90±1.01Cts, Endometrium: 15.81±0.92Cts, Whole Uterus: 17.28±1.63Cts; P<0.001) but no significant difference in expression was found between tissues supplying the smallest and the average fetus within a litter. These results confirm the spatial localisation of SSP1 within pig reproductive tissues, however its potential role in placental and fetal development warrants further research.

Reference: Hernandez, SC., Finlayson, HA., Ashworth, CJ., Haley, CS., and Archibald AL. 2009. Control of Pig Reproduction VIII, 117-118, Nottingham University Press (Funded by BPEX, BBSRC and Edinburgh University)

Notes

P79 A diet high in n-3 polyunsaturated fatty acids (PUFAs) in vivo modifies prostaglandin output from cultured ovine endometrium in vitro

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Uterine prostaglandin F2α (PGF2α) induces luteal regression in the cyclic ewe. Prostaglandins (PGs) of the 2-series, e.g. PGF2α, are derived from polyunsaturated fatty acids (PUFAs) through the sequential action of the phospholipases, cyclooxygenases (COX-1/2 where COX-1 is constitutively expressed and COX-2 is the inducible form) and terminal PG synthases (microsomal prostaglandin E synthase: mPGES-1, prostaglandin F synthase: PGH 9,11endoperoxide synthase: PGFS). We have previously demonstrated that a high n-3 PUFA diet delayed luteolysis through actions on PGF2α generation but were unable to clarify the mechanisms involved. Hence in this study we have addressed this question by culturing uterine explants from n-3 PUFA-fed ewes to assess PG generation (PGF2α, prostaglandin E2:PGE2) and to measure changes in components of PG synthesis (COX-1/2, mPGES-1 and PGFS). Welsh mountain ewes were individually fed a control diet or a diet high in n-3 PUFA for 6 weeks. To assess the impact of the diet on luteolysis, ewes were slaughtered during the late- luteal phase of the oestrous cycle (day 14, n=9). Endometrial explants were cultured for 24h in the presence/absence of oxytocin (OT). PGE2 and PGF2α in spent culture medium were assessed by specific radioimmunoassay. Protein and mRNA expression of COX-1/2, mPGES-1 and PGFS was determined by western blotting and qPCR respectively. The n-3 PUFA rich diet inhibited OT-stimulated PGF2α secretion ($P<0.05$) but had no effect on PGE2 secretion by cultured uterine explants. Expression of COX-2 protein and mRNA were unaffected by the n-3 PUFA diet. Similarly, mPGES-1 protein was also unaffected by the dietary intervention. However, PGFS mRNA was significantly decreased ($P<0.05$) in uterine explants of n-3 PUFA-fed ewes. Overall these results suggest that the n-3 PUFA diet delays luteal regression principally by inhibiting OT-induced PGF2α generation. This is achieved by decreasing expression of the terminal synthase, PGFS in the ovine uterus.

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